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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6:

C07K 7/00

(11) 11

(11) International Publication Number:

WO 99/07733

A2 |

(43) International Publication Date:

18 February 1999 (18.02.99)

(21) International Application Number:

PCT/CA98/00765

(22) International Filing Date:

10 August 1998 (10.08.98)

(30) Priority Data:

60/055,186

11 August 1997 (11.08.97)

US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: HEPATITIS C INHIBITOR PEPTIDES

(57) Abstract

Compound of formula (I) active against the Hepatitis C virus, wherein when Q is CH2, a is 0, b is 0 and B is an amide derivative; or when Q is N-Y wherein Y is H or C₁₋₆ alkyl, then B is an acyl derivative; R₆, when present, is C₁₋₆ alkyl substituted with carboxyl; R₅, when present, is C₁₋₆ alkyl optionally substituted with carboxyl;

when Q is either CH₂ or N-Y, then Z is oxo or thioxo; R_4 is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl); R_3 is C_{1-10} alkyl optionally substituted with carboxyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl); W is a proline derivative; R_1 ' is hydrogen, and R_1 is C_{1-6} alkyl optionally substituted with thiol; or R_1 is C_{2-6} alkenyl; or R_1 ' and R_1 together form a 3- to 6-membered ring; and A is hydroxy or a pharmaceutically acceptable salt or ester thereof.

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Hepatitis C Inhibitor Peptides

Field of the invention

The present invention relates to compounds, compositions and methods for the treatment of hepatitis C virus (HCV) infection. In particular, the present invention provides novel peptides and analogues thereof, pharmaceutical compositions containing such peptides and methods for using these peptides in the treatment of HCV infection.

Background of the invention

- Hepatitis C virus (HCV) is the major etiological agent of post-transfusion and community-acquired non-A non-B hepatitis worldwide. It is estimated that over 100 million people worldwide are infected by the virus. A high percentage of carriers become
- chronically infected and many progress to chronic liver disease, so called chronic hepatitis C. This group is in turn at high risk for serious liver disease such as liver cirrhosis, hepatocellular carcinoma and terminal liver disease leading to
- 25 death.

The mechanism by which HCV establishes viral persistence and causes a high rate of chronic liver disease has not been thoroughly elucidated. It is not known how HCV interacts with and evades the host immune system. In addition, the roles of cellular and humoral immune responses in protection against HCV infection and disease have yet to be established. Immunoglobulins have been reported for prophylaxis of transfusion-associated viral hepatitis. However, the

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Center for Disease Control does not presently recommend immunoglobulins for this purpose.

The lack of an effective protective immune response is hampering the development of a vaccine or adequate post-exposure prophylaxis measures, so in the nearterm, hopes are firmly pinned on antiviral interventions.

Various clinical studies have been conducted with the goal of identifying pharmaceutical agents capable of effectively treating HCV infection in patients afflicted with chronic hepatitis C. These studies have involved the use of interferon-alpha, alone and in combination with other antiviral agents. Such studies have shown that a substantial number of the participants do not respond to these therapies, and

of those that do respond favorably, a large proportion were found to relapse after termination of treatment.

Until recently, interferon (IFN) was the only available therapy of proven benefit approved in the clinic for patients with chronic hepatitis C. However the sustained response rate is low, and interferon treatment also induces severe side-effects (i.e. retinopathy, thyroiditis, acute pancreatitis, depression) that diminish the quality of life of treated patients. Recently, interferon in combination with ribavirin has been approved for patients non-responsive to IFN alone. However, the side effects caused by IFN are not alleviated with

this combination therapy.

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Therefore, a need exists for the development of effective antiviral agents for treatment of HCV infection that overcomes the limitations of existing pharmaceutical therapies.

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HCV is an enveloped positive strand RNA virus in the Flaviviridae family. The single strand HCV RNA genome is approximately 9500 nucleotides in length and has a single open reading frame (ORF) encoding a single 10 large polyprotein of about 3000 amino acids. In infected cells, this polyprotein is cleaved at multiple sites by cellular and viral proteases to produce the structural and non-structural (NS) In the case of HCV, the generation of 15 mature nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) is effected by two viral proteases. The first one, as yet poorly characterized, cleaves at the NS2-NS3 junction; the second one is a serine protease contained within the N-terminal region of 20 NS3 (henceforth referred to as NS3 protease) and mediates all the subsequent cleavages downstream of NS3, both in cis, at the NS3-NS4A cleavage site, and in trans, for the remaining NS4A-NS4B, NS4B-NS5A, NS5A-NS5B sites. The NS4A protein appears to serve 25 multiple functions, acting as a cofactor for the NS3 protease and possibly assisting in the membrane localization of NS3 and other viral replicase components. The complex formation of the NS3 protein with NS4A seems necessary to the processing events, 30 enhancing the proteolytic efficiency at all of the The NS3 protein also exhibits nucleoside triphosphatase and RNA helicase activities. a RNA-dependent RNA polymerase that is involved in the replication of HCV.

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A general strategy for the development of antiviral agents is to inactivate virally encoded enzymes that are essential for the replication of the virus. In this vein, patent application WO 97/06804 describes the (-) enantiomer of the nucleoside analogue cytosine-1,3-oxathiolane (also known as 3TC) as active against HCV. This compound, although reported as safe in previous clinical trials against HIV and HBV, has yet to be clinically proven active against HCV and its mechanism of action against the virus has yet to be reported.

Intense efforts to discover compounds which inhibit
the NS3 protease or RNA helicase of HCV have led to
the following disclosures:

- US patent 5,633,388 describes heterocyclicsubstituted carboxamides and analogues as being active against HCV. These compounds are directed against the helicase activity of the NS3 protein of the virus but clinical tests have not yet been reported.
- A phenanthrenequinone has been reported by Chu et
 al (Tet. Lett., (1996), 7229-7232) to have activity against the HCV NS3 protease in vitro.
 No further development on this compound has been reported.
- A paper presented at the Ninth International
 Conference on Antiviral Research, Urabandai,
 Fukyshima, Japan (1996) (Antiviral Research, 30,
 1, 1996; A23 (abstract 19)) reports thiazolidine
 derivatives to be inhibitory to the HCV protease.

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Several studies have reported compounds inhibitory to other serine proteases, such as human leukocyte elastase. One family of these compounds is reported in WO 95/33764 (Hoechst Marion Roussel, 1995). The peptides disclosed in that application are morpholinylcarbonyl-benzoyl-peptide analogues that are structurally different from the peptides of the present invention.

- o WO 98/17679 from Vertex Pharmaceuticals Inc.
 discloses inhibitors of serine protease,
 particularly, Hepatitis C virus NS3 protease.
 These inhibitors are peptide analogues based on
 the NS5A/5B natural substrate that contain Cterminal activated carbonyl function as an
 essential feature. These peptides were also
 reported to be active against other serine
 protease and are therefore not specific for HCV
 NS3 protease.
- o Hoffman LaRoche has also reported hexapeptides that are proteinase inhibitors useful as antiviral agents for the treatment of HCV infection. These peptides contain an aldehyde or a boronic acid at the C-terminus.
- o Steinkühler et al. and Ingallinella et al. have published on NS4A-4B product inhibition (Biochemistry (1998), 37, 8899-8905 and 8906-8914). These peptides and peptide analogues were published after the priority date of the present application.

One advantage of the present invention is that it provides peptides that are inhibitory to the NS3 protease of the hepatitis C virus.

A further advantage of one aspect of the present invention resides in the fact that these peptides specifically inhibit the NS3 protease and do not show significant inhibitory activity at concentrations up to 300 μ M against other serine proteases such as human leukocyte elastase (HLE), porcine pancreatic elastase (PPE), or bovine pancreatic chymotrypsin, or cysteine proteases such as human liver cathepsin B (Cat B).

Summary of the invention

We investigated peptides potentially inhibitory to the NS3 protease. The discovery that the N-terminal cleavage product (Ac-D-D-I-V-P-C-OH) of an analogue of a natural substrate of the NS3 protease was inhibitory led us to the peptide analogues of the present invention.

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Included in the scope of the invention are compounds of formula (I):

wherein Q is CH_2 or N-Y wherein Y is H or C_{1-6} alkyl;

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a) when $\bf Q$ is CH_2 , $\bf a$ is 0, $\bf b$ is 0, and $\bf B$ is an amide derivative of formula $\bf R_{11a}N(\bf R_{11b})$ -C(0)- wherein $\bf R_{11a}$ is H; C_{1-10} alkyl; C_6 aryl; C_{7-10} alkylaryl; C_{3-7} cycloalkyl optionally substituted with carboxyl; $(C_{3-7}$

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cycloalkyl)-(C_{1-6} alkyl); heterocycle- C_{1-6} alkyl such as

$$C_{1-4}$$
alkyl or C_{1-4} alkyl ;

and R_{11b} is C_{1-6} alkyl substituted with carboxyl, (C_{1-6} alkoxy) carbonyl or phenylmethoxycarbonyl; or C_{7-16} aralkyl substituted on the aromatic portion with carboxyl, (C_{1-6} alkoxy) carbonyl or phenylmethoxycarbonyl;

or R_{11a} and R_{11b} are joined to form a 3 to 7-membered nitrogen-containing ring optionally substituted with carboxyl or (C_{1-6} alkoxy) carbonyl; or

- b) when Q is N-Y, a is 0 or 1, b is 0 or 1, and B is an acyl derivative of formula R₁₁-C(O)-wherein R₁₁ is (i) C₁₋₁₀ alkyl optionally substituted with carboxyl, C₁₋₆ alkanoyloxy (e.g. AcOCH₂) or C₁₋₆ alkoxy (e.g. Boc); (ii) C₃₋₇ cycloalkyl optionally substituted with carboxyl, (C₁₋₆ alkoxy)carbonyl or
- phenylmethoxycarbonyl; (iii) C_{3-7} cycloalkyl substituted with carboxyl and one to three C_{1-6} alkyl substituents (iv) C_{4-10} (alkylcycloalkyl) optionally substituted on the cycloalkyl portion with carboxy, $(C_{1-6}$ alkoxy)carbonyl or phenylmethyoxycarbonyl; (v)

HOOC-(
$$C_{1-6}$$
alkyl)-N NCOO-(aryl or C_{1-6} alkylaryl) ,

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(v) C_6 or C_{10} aryl or $C_{7\text{--}16}$ aralkyl optionally substituted with $C_{1\text{--}6}$ alkyl;

 \mathbf{R}_{6} , when present, is C_{1-6} alkyl substituted with carboxyl;

5 \mathbf{R}_5 , when present, is C_{1-6} alkyl optionally substituted with carboxyl;

or

when Q is either CH_2 or N-Y;

c) R_4 is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10}

10 (alkylcycloalkyl);

Z is oxo or thioxo;

 ${f R_3}$ is $C_{1\text{--}10}$ alkyl optionally substituted with carboxyl, $C_{3\text{--}7}$ cycloalkyl or $C_{4\text{--}10}$ (alkylcycloalkyl); ${f W}$ is a group of formula II:

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wherein $\mathbf{R_2}$ is C_{1-10} alkyl or C_{3-10} cycloalkyl optionally substituted with carboxyl; C_6 or C_{10} aryl or C_{7-16} aralkyl; or

20 W is a group of formula II':

wherein X is CH or N; and

R₂' is divalent C₃₋₄ alkylene which together with X and the carbon atom to which X and R₂, are attached form a 5- or 6-membered ring, said ring optionally substituted with OH; SH; NH₂; carboxyl; R₁₂; OR₁₂, C(0)OR₁₂, SR₁₂, NHR₁₂ or NR₁₂R₁₂' wherein R₁₂ and R₁₂'are independently:

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cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl, said alkyl or alkenyl optionally substituted with NH2, OH, SH, halo, or carboxyl; said alkyl 5 or alkenyl optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N; or $\mathbf{R_{12}}$ and $\mathbf{R_{12}}'$ are independently C_6 or C_{10} aryl or C_{7-16} aralkyl optionally substituted with C_{1-6} 10 alkyl, CF3, NH2, OH, SH, halo, carboxyl, C1-6 alkyl substituted with carboxyl or phenyl optionally substituted with C₁₋₆ alkyl, C₁₋₆ alkoxy, halo, acetylamido or nitro; said aryl or aralkyl optionally containing at least one 15 heteroatom selected independently from the group consisting of: 0, S, and N; said cyclic alkyl, cyclic alkenyl, aryl or aralkyl being optionally fused with a second 5-, 6-, or 7-membered ring to form a cyclic system 20 or heterocyclic system, said second ring being optionally substituted with NH2, OH, SH, halo, carboxyl or carboxy(lower)alkyl; said second ring optionally containing at least one heteroatom selected independently from the group 25 consisting of: 0, S, and N; or X is CH or N; and R_2 , is a divalent C_{3-4} alkylene which together with X and the carbon atom to which X and R_{2} , are attached form a 5- or 6-membered ring which in turn is fused with a second 5-, 6- or 7-30 membered ring to form a cyclic system wherein the second ring is substituted with OR12.. wherein R12.. is C₇₋₁₆ aralkyl;

 R_1 ' is hydrogen, and R_1 is C_{1-6} alkyl optionally substituted with thiol or halo; or R_1 is C_{2-6} alkenyl; or

 R_1 ' and R_1 together form a 3- to 6-membered ring optionally substituted with C_{1-6} alkyl; and A is hydroxy or a pharmaceutically acceptable salt or ester thereof.

Included within the scope of this invention is a

pharmaceutical composition comprising an antihepatitis C virally effective amount of a compound of
formula I, or a therapeutically acceptable salt or
ester thereof, in admixture with a pharmaceutically
acceptable carrier medium or auxiliary agent.

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An important aspect of the invention involves a method of treating a hepatitis C viral infection in a mammal by administering to the mammal an antihepatitis C virally effective amount of the compound of formula I, or a therapeutically acceptable salt or ester thereof or a composition as described above.

Another important aspect involves a method of inhibiting the replication of hepatitis C virus by exposing the virus to a hepatitis C viral NS3 protease inhibiting amount of the compound of formula I, or a therapeutically acceptable salt or ester thereof or a composition as described above.

30 Still another aspect involves a method of treating a hepatitis C viral infection in a mammal by administering thereto an anti-hepatitis C virally effective amount of a combination of the compound of formula I, or a therapeutically acceptable salt or ester thereof, and an interferon. A pharmaceutical

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composition comprising the combination in admixture with a pharmaceutically acceptable carrier medium or auxiliary agent is also within the scope of this invention.

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Detailed description of the invention

As used herein, the following definitions apply unless otherwise noted:

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With reference to the instances where (R) or (S) is used to designate the configuration of a radical, e.g. R_4 of the compound of formula I, the designation is done in the context of the compound and not in the context of the radical alone.

The natural amino acids, with exception of glycine, contain a chiral carbon atom. Unless otherwise specifically indicated, the compounds containing natural amino acids with the L-configuration are preferred. However, applicants contemplate that when specified, some amino acids of the formula I can be of either D- or L- configuration or can be mixtures of D- and L-isomers, including racemic mixtures.

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The designation "P1, P2, P3 et." as used herein refer to the position of the amino acid residues starting from the C-terminus end of the peptide analogues and extending towards the N-terminus (i.e. P1 refers to position 1 from the C-terminus, P2: second position from the C-terminus, etc.) (see Berger A. & Schechter I., Transactions of the Royal Society London series B257, 249-264 (1970)).

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The abbreviations for the $\alpha\text{-amino}$ acids are set forth in Table A.

Table A

AMINO ACID	SYMBOL
Allylglycine	AlGly
Aminobutyric acid	Abu
1-aminocyclopentyl-	Acpe
carboxylic acid	
1-aminocyclopropyl-	Acca
carboxylic acid	ĺ
Alanine	Ala
Aspartic acid	Asp
Cysteine	Cys
Cyclohexylalanine	Cha
Cyclohexylglycine	Chg
(also named: 2-amino-2- cyclohexylacetic acid)	
Glutamic acid	Glu
Isoleucine	Ile
Leucine	Leu
Norvaline	Nva
Phenylalanine	Phe
Pipecolic acid	Pip
Proline	Pro
4(R)-Hydroxyproline	Нур
4(R)-Benzyloxyproline	Hyp(4-Bn)
Valine	Val
tert-Butylglycine	Tbg

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As used herein the term "aminobutyric acid" refers to a compound of formula:

As used herein the term "allylglycine" refers to a compound of formula:

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As used herein the term "1-aminocyclopropyl-carboxylic acid" (Acca) refers to a compound of formula:

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As used herein the term "tert-butylglycine" refers to a compound of formula:

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The term "residue" with reference to an amino acid or amino acid derivative means a radical derived from the corresponding α -amino acid by eliminating the hydroxyl of the carboxy group and one hydrogen of the α -amino group. For instance, the terms Gln, Ala, Gly, Ile, Arg, Asp, Phe, Ser, Leu, Cys, Asn, Sar and Tyr represent the "residues" of L-glutamine, L-alanine, glycine, L-isoleucine, L-arginine, L-aspartic acid,

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L-phenylalanine, L-serine, L-leucine, L-cysteine, L-asparagine, sarcosine and L-tyrosine, respectively.

The term "side chain" with reference to an amino acid or amino acid residue means a group attached to the α -carbon atom of the α -amino acid. For example, the R-group side chain for glycine is hydrogen, for alanine it is methyl, for valine it is isopropyl. For the specific R-groups or side chains of the α -amino acids reference is made to A.L. Lehninger's text on Biochemistry (see chapter 4).

The term "halo" as used herein means a halogen radical selected from bromo, chloro, fluoro or iodo.

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The term "C₁₋₆ alkyl" or "(lower)alkyl" as used herein, either alone or in combination with another radical, means straight chain or branched alkyl radicals containing up to six carbon atoms and includes, for example, methyl, ethyl, propyl, butyl, hexyl, 1-methylethyl, 1-methylpropyl, 2-methylpropyl, 1,1-dimethylethyl.

Likewise, the terms C_{1-3} alkyl" C_{1-4} alkyl" and C_{1-10} alkyl" are used to denote alkyl radials containing up to three, four and ten carbon atoms, respectively.

The term "C₃₋₇ cycloalkyl" as used herein, either alone or in combination with another radical, means a cycloalkyl radical containing from three to seven carbon atoms and includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.

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The term " C_{4-10} (alkylcycloalkyl) as used herein means a cycloalkyl radical containing from three to seven carbon atoms linked to an alkyl radical, the linked radicals containing up to ten carbon atoms; for example, cyclopropylmethyl, cyclopentylethyl, cyclohexylmethyl, cyclohexylethyl or cycloheptylethyl.

The term "C₂₋₁₀ alkenyl" as used herein, either alone or in combination with another radical, means an alkyl radical as defined above containing from 2 to 10 carbon atoms, and further containing at least one double bond. For example alkenyl includes allyl.

The term "C₃₋₄ alkylene" as used herein means a divalent alkyl radical derived by the removal of two hydrogen atoms from a straight or branched chain aliphatic hydrocarbon containing from three to four carbon atoms and includes, for example, -CH₂CH₂CH₂-,

 $CH(CH_3)CH_2CH_2-$, $-CH_2C(CH_3)_2-$ and $-CH_2CH_2CH_2-$.

The term " C_{1-6} alkoxy" as used herein, either alone or in combination with another radical, means the radical $-0-C_{1-6}$ alkyl wherein alkyl is as defined above containing up to six carbon atoms. Alkoxy includes methoxy, ethoxy, propoxy, 1-methylethoxy, butoxy and 1,1-dimethylethoxy. The latter radical is known commonly as tert-butoxy.

The term "C₆ or C₁₀ aryl" as used herein, either alone or in combination with another radical, means either an aromatic monocyclic system containing 6 carbon atoms or an aromatic cyclic system containing 10

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carbon atoms. For example, aryl includes phenyl or naphthalene.

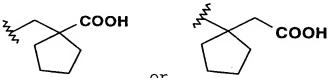
The term "C₇₋₁₆ aralkyl" as used herein, either alone or in combination with another radical, means an aryl as defined above linked through an alkyl group, wherein alkyl is as defined above containing from 1 to 6 carbon atoms. Aralkyl includes for example benzyl, and butylphenyl.

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The term "carboxy(lower)alkyl" as used herein, either alone or in combination with another radical, means a carboxyl group (COOH) linked through a (lower)alkyl group as defined above and includes for example butyric acid or the groups:



The term "cyclic" or "cyclic system" as used herein, 20 either alone or in combination with another radical, means a monovalent radical derived by removal of a hydrogen from a saturated or unsaturated cyclic hydrocarbon, containing from three to seven carbon atoms, unless otherwise indicated and optioonally 25 conctaing one or more heteroatom. The term cyclic or cyclic system includes, for example, cyclopropane, cyclopentane, cyclohexane, cyclohexene, decalin, tetralin, indene, and naphthalene.

30 The term "heterocycle" as used herein, either alone or in combination with another radical, means a

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monovalent radical derived by removal of a hydrogen from a five-, six-, or seven-membered saturated or unsaturated heterocycle containing from one to four heteroatoms selected from nitrogen, oxygen and sulfur. Examples of suitable heterocycles include: pyrrolidine, tetrahydrofuran, thiazolidine, pyrrole, thiophene, diazepine, 1H-imidazole, 1-methyl-1H-imidazole, isoxazole, thiazole, 2-methylthiazole, 2-aminothiazole, piperidine, 1,4-dioxane, 4-morpholine, pyridine, 2-methylpyridine, pyrimidine, 4-methylpyrimidine and 2,4-dimethylpyrimidine.

The term "heterocyclic system" as used herein, either alone or in combination with another radical, means a heterocycle as defined above fused to one or more other cycle be it a heretocycle or any other cycle. Examples of suitable heterocyclic systems include: thiazolo[4,5-b]-pyridine, quinoline, or indole.

The term "pharmaceutically acceptable ester" as used herein, either alone or in combination with another radical, means esters of the compound of formula I in which any of the carboxyl functions of the molecule, but preferably the carboxy terminus, is replaced by an alkoxycarbonyl function:



in which the **R** moiety of the ester is selected from alkyl (e.g. methyl, ethyl, n-propyl, t-butyl, n-butyl); alkoxyalkyl (e.g. methoxymethyl); alkoxyacyl (e.g. acetoxymethyl); aralkyl (e.g. benzyl); aryloxyalkyl (e.g. phenoxymethyl); aryl (e.g. phenyl), optionally substituted with halogen, C₁₋₄ alkyl or C₁₋₄ alkoxy. Other suitable prodrug esters

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can be found in Design of prodrugs, Bundgaard, H. Ed. Elsevier (1985) incorporated herewith by reference. Such pharmaceutically acceptable esters are usually hydrolyzed *in vivo* when injected in a mammal and transformed into the acid form of the compound of formula I.

The term "pharmaceutically acceptable salt" as used herein includes those derived from pharmaceutically acceptable bases. Examples of suitable bases include choline, ethanolamine and ethylenediamine. Na[†], K[†], and Ca^{††} salts are also contemplated to be within the scope of the invention (also see Pharmaceutical salts, Birge, S.M. et al., J. Pharm. Sci. (1977), 66, 1-19, incorporated herein by reference).

Preferred embodiments

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A further preferred group of compounds are represented by formula Ia:

wherein Y is H or C_{1-6} alkyl;

a is 0 or 1;

b is 0 or 1;

B is an acyl derivative of formula R_{11} -C(0)-wherein R_{11} is (i) C_{1-10} alkyl optionally substituted with carboxyl, C_{1-6} alkanoyloxy or C_{1-6} alkoxy; (ii) C_{3-7} cycloalkyl optionally substituted with carboxyl, (C_{1-6} alkoxy)carbonyl or phenylmethoxycarbonyl; (iii) C_{3-7}

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cycloalkyl substituted with carboxyl and one to three C_{1-6} alkyl substituents (iv) C_{4-10} (alkylcycloalkyl) optionally substituted on the cycloalkyl portion with carboxy, (C_{1-6} alkoxy)carbonyl or

5 phenylmethyoxycarbonyl; (v)

HOOC-(
$$C_{1-6}$$
alkyl)-N NCOO-(aryl or C_{1^-6} alkylaryl) ,

HO
$$(CH_2)_{1-5}$$
 or O

(v) C_6 or C_{10} aryl or C_{7-16} aralkyl optionally substituted with C_{1-6} alkyl;

 R_6 , when present, is C_{1-6} alkyl substituted with carboxyl;

 $R_{5}, \mbox{ when present, is } C_{1\text{-}6} \mbox{ alkyl optionally substituted}$ with carboxyl; and

 \mathbf{R}_4 is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl);

15 R_3 , W, R_1 , R_1 ' and A are as defined above.

Preferably, **B** is an acyl derivative of formula $\mathbf{R_{11}}$ C(O) - wherein $\mathbf{R_{11}}$ is: C_{1-6} alkyl optionally substituted with carboxyl, C_{1-6} alkanoyloxy or C_{1-6}

20 alkoxy;

 C_{3-7} cycloalkyl optionally substituted with carboxyl, MeOC(0), EtOC(0) or BnOC(0);

3-carboxypropionyl (DAD) or 4-carboxybutyryl (DAE); or

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More preferably, $\bf B$ is acetyl, 3-carboxypropionyl, 4-carboxylbutyryl, AcOCH₂C(O), Me₃COC(O),

$$C(O)OH$$
 $C(O)OBn$
 $C(O)OH$
 $C(O)OBn$
 $C(O)OH$
 $C(O)OBn$
 $C(O)OH$
 $C(O)OBn$
 $C(O)OBn$

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Still, more preferably, **B** is acetyl, 3-carboxypropionyl (DAD), 4-carboxybutyryl (DAE), AcOCH₂C(O),

$$C = 0$$
or
$$C(O)OH$$

$$C(O)OBn$$

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Most preferably, **B** is acetyl.

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Preferably, \mathbf{R}_{6} , when present, is the side chain of Asp or Glu.

Most preferably, $\mathbf{R_6}$, when present, is the side chain of Asp.

5 Alternatively, preferably, ${\bf a}$ is 0 and then ${\bf R_6}$ is absent.

Preferably, R_5 , when present, is the side chain of an amino acid selected from the group consisting of: D-

10 Asp, L-Asp, D-Glu, L-Glu, D-Val, L-Val, D-tert-butylglycine (Tbg), and L-Tbg.

More preferably, R_5 , when present, is the side chain of D-Asp, D-Val, or D-Glu.

Most preferably, R_5 , when present, is the side chain of D-Glu.

Alternatively, preferably ${\bf a}$ is 0 and ${\bf b}$ is 0, and then both ${\bf R}_6$ and ${\bf R}_5$ are absent.

Alternatively, another preferred group of compounds are represented by formula (Ib):

wherein **B** is preferably an amide of formula $R_{11a}N(R_{11b})C(0)$ - wherein R_{11a} is preferably C_{1-6} alkyl, C_{3-6} cycloalkyl, C_{3-7} (alkylcylcoalkyl) optionally substituted with carboxy, C_{1-3} carboxyalkyl, C_6 aryl, C_{7-10} arylalkyl, 2-tetrahydrofuranylmethyl, or 2-thiazolidylmethyl; and R_{11b} is preferably C_{1-4} alkyl substituted with carboxyl.

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Most preferably, R_{11a} is cyclopropylmethyl, isopropyl, carboxyethyl, benzylmethyl, benzyl, or 2-tetrahydrofuranylmethyl. More preferably R_{11b} is C_{1-4} alkyl substituted with carboxyl. Most preferably, R_{11b} is ethyl carboxyl.

Compounds of the invention include compounds of formula I wherein, preferably, R_4 is selected from the group consisting of: isopropyl, cyclohexyl, tertbutyl, 1-methylpropyl, and 2-methylpropyl.

More preferably, R_4 is cyclohexyl or 1-methylpropyl.

Most preferably, R_4 is cyclohexyl.

Compounds of the invention include compounds of formula I wherein **Z** is preferably oxo.

Compounds of the invention include compounds of formula I wherein preferably, R_3 is the side chain of an amino acid selected from the group consisting of: Ile, allo-Ile, Chg, Cha, Val, Tbg or Glu.

More preferably, R_3 is the side chain of Val, Tbg or Chg.

Most preferably, $\mathbf{R_3}$ is the side chain of Val. 25

Compounds of the invention include compounds of formula I wherein preferably, \mathbf{W} is a group of formula II:

30 wherein $\mathbf{R_2}$ is C_{1-8} alkyl; C_{1-8} alkyl substituted with carboxyl, C_{1-6} alkoxycarbonyl, benzyloxycarbonyl or

benzylaminocarbonyl; C₃₋₇ cycloalkyl or benzyl.

Preferably, R₂ is the side of chain of Abu, Leu, Phe,
Cha, Val, Ala, Asp, Glu, Glu(Obn), or Glu(NHBn).

Most preferably, R₂ is the side chain of Asp,
aminobutyric acid (Abu) or Val.

Still, more preferably, compounds of the invention include compounds of formula I wherein \boldsymbol{W} is a group of formula II':

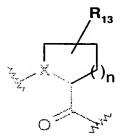
$$X \xrightarrow{\mathbf{R}_{2}^{\mathbf{I}}}$$

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wherein preferably, X is CH or N.

More preferably $\mathbf{R_2'}$ is a C_3 or C_4 alkylene (shown in bold) that joins \mathbf{X} to form a 5- or 6-membered ring of formula III:



Formula III

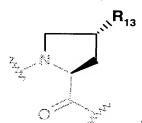
 R_{2} ' being optionally substituted at any position with 20 R_{13} , wherein X is CH or N; n is 1 or 2, and R_{13} is as defined below.

Most preferably, \mathbf{X} is N. For example, preferably $\mathbf{R_2'}$ is propyl joined to \mathbf{X} wherein \mathbf{X} is nitrogen to form a proline substituted with $\mathbf{R_{13}}$ at P2.

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Most preferably R_2 ' is the side chain of proline substituted at the 3-, 4-, or 5-position with R_{13} , wherein R_{13} is as defined below.

Still, most preferably R_2 ' is the side chain of proline (as shown in bold) substituted with R_{13} at the 4-position with the stereochemistry shown in formula III':



Formula III′

wherein R_{13} is preferably OH; SH; NH₂; carboxyl; R_{12} ; OR₁₂, SR₁₂, NHR₁₂ or NR₁₂R₁₂' wherein R₁₂ and R₁₂' are independently:

cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl, said alkyl or alkenyl optionally substituted with NH_2 , OH, SH, halo, or carboxyl; said alkyl or alkenyl optionally containing at least one heteroatom independently selected from the group consisting of: O, S, and N; or

R₁₂ and R₁₂, are independently C₆ or C₁₀ aryl or C₇₋₁₆ aralkyl optionally substituted with C₁₋₆ alkyl, NH₂, OH, SH, halo, carboxyl or carboxy(lower)alkyl; said aryl or aralkyl optionally containing at least one heteroatom independently selected from the group consisting of: O, S, and N; said cyclic alkyl, cyclic alkenyl, aryl or

aralkyl being optionally fused with a second 5-, 6-, or 7-membered ring to form a cyclic system

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or heterocyclic system, said second ring being optionally substituted with NH₂, OH, SH, halo, carboxyl or carboxy(lower)alkyl; said second ring optionally containing at least one heteroatom independently selected from the group consisting of: O, S, and N.

More preferably, R₁₃ is OR₁₂ or SR₁₂ wherein R₁₂ is a C₆ or C₁₀ aryl or C₇₋₁₆ aralkyl, said first aryl or aralkyl optionally substituted with C₁₋₆ alkyl, C₃₋₇ cycloalkyl, NH₂, OH, SH, halo, C₁₋₆ alkoxy, carboxyl, carboxy(lower)alkyl, or a second aryl or aralkyl; said first and second aryl or aralkyl optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N.

Most preferably, R₁₃ is Bn; PhCH₂CH₂; PhCH₂CH₂; O-Bn; o-tolylmethoxy; m-tolylmethoxy; p-tolylmethoxy;

1-naphtyloxy; 2-naphtyloxy; 1-naphthalenylmethoxy; 2-naphthalenylmethoxy; (4-tert-butyl)methoxy; (3I-Ph)CH₂O; (4Br-Ph)O; (2Br-Ph)O; (3Br-Ph)O; (4I-Ph)O; (3Br-Ph)CH₂O; (3,5-Br₂-Ph)CH₂O;

Still most preferably, R₁₃ is PhCH₂CH₂CH₂; O-Bn; 1-naphtyloxy; 2-naphtyloxy; 1-naphthalenylmethoxy; 2-naphthalenylmethoxy;

Further include within the invention are compounds of formula I wherein R_1 ' is preferably hydrogen and R_1 is C_{1-6} alkyl optionally substituted with thiol. For example, R_1 is preferably the side chain of the amino acid selected from the group consisting of: cysteine (Cys), aminobutyric acid (Abu), norvaline (Nva), or allylglycine (AlGly).

More preferably, R_1 ' is H and R_1 is propyl. For example, R_1 is more preferably the side chain of the

Alternatively, preferably, R₁' and R₁ together form a 3- to 6-membered ring, said ring being optionally substituted with ethyl. For example, R₁'and R₁ together form preferably a cyclopropyl, a cyclobutyl, a cyclopentyl, or a cyclohexyl ring. Alternatively, more preferably, R₁' and R₁ together form a cyclopropyl, For example, R₁' and R₁ together can be the side chain (shown in bold) of the following amino acid:

25 referred to as 1-aminocyclopropylcarboxylic acid (Acca).

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amino acid Nva.

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Further included in the present invention are compounds of fomrula I wherein A is preferably hydroxy, a salt or an ester thereof. More preferably, A is hydroxy or an ester thereof. Most preferably, A is hydroxy.

More preferably, the ester is C_{1-6} alkoxy, or (aryl C_{1-6} -alkoxy). Most preferably, the ester is methoxy, ethoxy, phenoxy, or benzyloxy

Included in the scope of the invention are compounds of formula I wherein $\bf Q$ is CH_2 , $\bf a$ is 0, $\bf b$ is 0, and then $\bf B$ is an amide of formula $\bf R_{11a}N(\bf R_{11b})-C(\bf 0)-$ wherein

15 R_{11a} is C₁₋₆ alkyl, C₃₋₆ cycloalkyl, C₃₋₇
(alkylcylcoalkyl) optionally substituted with
carboxy, C₁₋₃ carboxyalkyl, phenyl, C₇₋₁₀ arylalkyl,
2-tetrahydrofuranylmethyl, or 2-thiazolidylmethyl;
and R_{11b} is phenyl; or C₁₋₆ alkyl substituted with

20 carboxyl or C_{1-4} carboxyalkyl;

or

Q is N-Y wherein Y is H or C_{1-6} alkyl; **a** is 0 or 1; **b** is 0 or 1; and **B** is an acyl derivative of formula R_{11} -C(0)- wherein R_{11} is (i) C_{1-6} alkyl, C_{1-6} alkyl

substituted with carboxyl, MeC(0)0-, MeO-, EtO-, MeCH₂CH₂O- or Me₃C-O-; (ii) cyclopentyl or cyclohexyl optionally substituted with carboxyl; (iv) C₄₋₁₀ (alkylcycloalkyl) optionally substituted on the cycloalkyl portion with carboxyl;

30 (v)

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(vi) phenyl, benzyl or phenylethyl;

 R_6 , when present, is CH_2COOH or CH_2CH_2COOH , R_5 , when present, is C_{1-6} alkyl or CH_2COOH or CH_2CH_2COOH ;

and when ${\bf Q}$ is either CH_2 or $N-{\bf Y}$, ${\bf R}_4$ is C_{1-6} alkyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl);

10 **Z** is oxo or thio;

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 R_3 is C_{1-6} alkyl; C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl);

W is a group of formula II wherein $\mathbf{R_2}$ is C_{1-10} alkyl, C_{3-10} cycloalkyl, C_{7-11} aralkyl; CH_2COOH or CH_2CH_2COOH ;

- or W is a group of formula II' wherein X is N or CH and R₂, is the divalent radical -CH₂CH₂CH₂- or CH₂CH₂CH₂- which together with X and the carbon atom to which X and R₂, are attached form a 5- or 6-membered ring, said ring optionally substituted with
- OR₁₂, C(0)OR₁₂, SR₁₂, NHR₁₂ or NR₁₂R₁₂, wherein R₁₂ and R₁₂, are independently:

cyclic C₃₋₁₆ alkyl or acyclic C₁₋₁₆ alkyl or cyclic C₃₋₁₆ alkenyl or acyclic C₂₋₁₆ alkenyl, said alkyl or alkenyl optionally substituted

with NH₂, OH, SH, halo, or carboxyl; said alkyl or alkenyl optionally containing at least one heteroatom independently selected from the grop consisting of: O, S, and N; or R₁₂ and R₁₂, are independently C₆ or C₁₀ aryl or C₇₋₁₆ aralkyl optionally substituted with C₁₋₆ alkyl, CF₃, NH₂, OH, SH, halo, carboxyl, C₁₋₆ alkyl substituted with carboxyl, or phenyl optionally substituted with C₁₋₆ alkyl, C₁₋₆ alkoxy or halo; said aryl or aralkyl optionally containing at least one

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heteroatom independently selected from the group consisting of: 0, S, and N; said cyclic alkyl, cyclic alkenyl, aryl or aralkyl being optionally fused with a second 5-, 6-, or 7-membered ring to form a cyclic system or heterocyclic system, said second ring being optionally substituted with NH_2 , OH, SH, halo, carboxyl or C_{1-6} alkyl substituted with carboxyl; said second ring optionally containing at least one heteroatom independently selected from the group consisting of: 0, S, and N; or X is N; and R_2 , is - $CH_2CH_2CH_2$ - or $-CH_2CH_2CH_2CH$ - which together with ${\bf X}$ and the carbon atom to which \boldsymbol{x} and $\boldsymbol{R_2}$, are attached form a 5- or 6-membered ring, which in turn is fused to a phenyl to form a cyclic system wherein the phenyl ring is substituted with OR_{12} wherein R_{12} is phenylmethyl or phenylethyl;

R₁, is hydrogen and R₁ is methyl, thiomethyl, 120 methylethyl, propyl, 1-methylpropyl, 2(methylthio)ethyl or 2-propylene; or R₁, and R₁
together with the carbon atom to which they are
attached form a cyclopropyl which may optionally be
substituted with ethyl; and

A is hydroxy or a pharmaceutically acceptable salt thereof; C_{1-6} alkoxy, or (aryl C_{1-6} -alkoxy).

Included in the scope of the invention are compounds of formula Ia, wherein **B** is an acyl derivative of formula **R**₁₁-C(0) - wherein **R**₁₁ is C₁₋₆ alkoxy, C₁₋₁₀ alkyl optionally substituted with carboxyl; C₃₋₇ cycloalkyl optionally substituted with carboxyl or benzylcarboxy; or

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R₆ is absent;

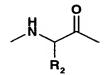
R₅ is absent;

 R_4 is $C_{1\text{--}10}$ alkyl, $C_{3\text{--}7}$ cycloalkyl or $C_{4\text{--}10}$

5 (alkylcycloalkyl);

 $\mathbf{R_3}$ is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl);

W is a group of formula II:



Formula II

wherein $\mathbf{R_2}$ is C_{1-6} alkyl; C_{3-6} cycloalkyl; C_{1-6} alkyl substituted with carboxyl; C_6 or C_{10} aryl; or C_{7-11} aralkyl;

or

W is a group of formula II':

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Formula II'

wherein \boldsymbol{x} is N; and $\boldsymbol{R_2}$, is as defined in claim 1, and

A is hydroxy or a pharmaceutically acceptable salt thereof; methoxy, ethoxy, phenoxy, or benzyloxy.

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Included in the scope of the invention are compounds of formula Ia, wherein ${\bf B}$ is acetyl, 3-carboxypropionyl, 4-carboxylbutyryl, AcOCH₂C(O), Me₃COC(O),

Y is H or Me, a is 0 or 1, b is 0 or 1,

 R_6 , when present, is the side chain of Asp or Glu,

 R_5 , when present, is the side chain of Asp, D-Asp,

5 Glu, D-Glu, Val, D-Val or Tbg,

 $\mathbf{R_4}$ is the side chain of Val, Chg, Tbg, Ile or Leu, \mathbf{Z} is oxo or thioxo,

 R_3 is hydrogen or the side chain of Ile, Chg, Val, Glu;

W is Abu, Leu, Phe, Val, Ala, Glu, Glu(OBn); or
W is group of formula III':

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wherein R₁₃ is Bn, PhCH₂CH₂, PhCH₂CH₂CH₂, O-Bn, o-tolylmethoxy, m-tolylmethoxy, p-tolylmethoxy, 1-naphthalenylmethoxy, 2-naphthalenylmethoxy, (4-tert-butyl)benzyloxy, (3I-Ph)CH₂O, (4Br-Ph)O, (2Br-Ph)O, (3Br-Ph)O, (3Br-Ph)CH₂O, (3,5-Br₂-Ph)CH₂O,

 $\mathbf{R_{1}}$, is H and $\mathbf{R_{1}}$ is the side chain of Cys, Abu, Nva or allylglycine; or

 $\mathbf{R_{1}}$, and $\mathbf{R_{1}}$ together with the carbon atom to which they are attached form a cyclopropyl; and \mathbf{A} is hydroxyl.

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Also included in the scope of the invention are compounds of formula Ib, wherein **B** is an amide of formula $R_{11a}N(R_{11b})-C(0)$ wherein R_{11a} is C_{1-6} alkyl, C_{3-6} cycloalkyl, C_{3-7} (alkylcylcoalkyl) optionally

substituted with carboxy, C_{1-3} carboxyalkyl, phenyl, C_{7-10} arylalkyl,

2-tetrahydrofuranylmethyl, or 2-thiazolidylmethyl; and $\mathbf{R_{11b}}$ is phenyl; or C_{1-6} alkyl substituted with carboxyl or C_{1-4} carboxyalkyl;

15 R₄ is cyclohexyl;

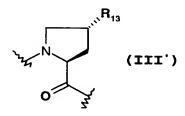
Z is oxo;

 $\mathbf{R_3}$ is hydrogen or the side chain of Ile, Chg, Val, Glu:

W is Abu, Leu, Phe, Val, Ala, Glu, Glu(OBn); or

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W is group of formula III':



wherein R₁₃ is Bn, PhCH₂CH₂, PhCH₂CH₂CH₂, O-Bn, o-tolylmethoxy, m-tolylmethoxy, p-tolylmethoxy, 1-naphthalenylmethoxy, 2-naphthalenylmethoxy, (4-tert-butyl)methoxy, (3I-Ph)CH₂O, (4Br-Ph)O, (2Br-Ph)O, (3Br-Ph)O, (4I-Ph)O, (3Br-Ph)CH₂O, (3,5-Br₂-Ph)CH₂O,

 $R_{1}\text{,}$ is H and R_{1} is the side chain of Cys, Abu, Nva or allylglycine; or

5 R_1 , and R_1 together with the carbon atom to which they are attached form a cyclopropyl; and A is hydroxyl.

Also included within the scope of the present invention are compounds of formula I:

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wherein **B** is an acyl derivative of formula R_{11} -C(O)-wherein R_{11} is C_{1-10} alkyl optionally substituted with carboxyl; C_{3-7} cycloalkyl optionally substituted with carboxyl; or a C_{4-10} (alkylcycloalkyl) optionally substituted on the cycloalkyl portion with carboxyl; or R_{11} is C_6 or C_{10} aryl or C_{7-16} aralkyl optionally substituted with a C_{1-6} alkyl

a is 0 or 1;

 \mathbf{R}_{6} , when present, is C_{1-6} alkyl optionally substituted with carboxvl;

b is 0 or 1;

 \mathbf{R}_{5} , when present, is C_{1-6} alkyl optionally substituted with carboxyl;

Q is N-Y wherein Y is H or C_{1-6} alkyl;

15 \mathbf{R}_4 is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl);

Z is oxo;

 $\mathbf{R_3}$ is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl);

20 W is a group of formula II:

Formula II

wherein $\mathbf{R_2}$ is C_{1-6} alkyl; C_{1-6} alkyl optionally substituted with carboxyl; C_6 or C_{10} aryl; or C_{7-16} aralkyl;

W is a group of formula II':

wherein X is CH or N; and

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 R_2 ' is C_{3-4} alkyl that joins X to form a 5- or 6-membered ring, said ring optionally substituted with OH; SH; NH_2 ; carboxyl; R_{12} ; OR_{12} , SR_{12} , NHR_{12} or $NR_{12}R_{12}$ ' wherein R_{12} and R_{12} ' are independently:

cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl, said alkyl or alkenyl optionally substituted with NH_2 , OH, SH, halo, or carboxyl; said alkyl or alkenyl optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N; or

 R_{12} and R_{12} ' are independently C_6 or C_{10} aryl or C_{7-16} aralkyl optionally substituted with C_{1-6} alkyl, NH_2 , OH, SH, halo, carboxyl or C_{1-6} alkyl substituted with carboxyl; said aryl or aralkyl optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N;

said cyclic alkyl, cyclic alkenyl, aryl or aralkyl being optionally fused with a second 5-, 6-, or 7-membered ring to form a cyclic system or heterocyclic system, said second ring being optionally substituted with NH₂, OH, SH, halo, carboxyl or carboxy(lower)alkyl; said second ring optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N;

and

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 R_1 ', is hydrogen, and R_1 is C_{1-6} alkyl optionally substituted with thiol, or C_{2-6} alkenyl; or R_1 ' and R_1 together form a 3- to 6-membered ring optionally substituted with C_{1-6} alkyl; and A is OH or a pharmaceutically acceptable salt or ester thereof.

Finally, included in the scope of the invention are all compounds of formula I presented in Tables 1 to 4.

According to an alternate embodiment, the pharmaceutical compositions of this invention may additionally comprise an antiviral agent. Examples of antiviral agents include, ribavirin and amantadine.

According to another alternate embodiment, the pharmaceutical compositions of this invention may additionally comprise other inhibitors of HCV protease.

According to yet another alternate embodiment, the pharmaceutical compositions of this invention may additionally comprise an inhibitor of other targets in the HCV life cycle, such as helicase, polymerase, or metalloprotease.

The pharmaceutical compositions of this invention may be administered orally, parenterally or via an implanted reservoir. We prefer oral administration or administration by injection. The pharmaceutical compositions of this invention may contain any conventional non-toxic pharmaceutically-acceptable

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carriers, adjuvants or vehicles. In some cases, the pH of the formulation may be adjusted with pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intra-articular, intrasynovial, intrasternal, intrathecal, and intralesional injection or infusion techniques.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example. Tween 80) and suspending agents.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable 20 dosage form including, but not limited to, capsules, tablets, and aqueous suspensions and solutions. the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are 25 also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are administered orally, the active ingredient is combined with emulsifying and suspending agents. 30 desired, certain sweetening and/or flavoring and/or coloring agents may be added.

Other suitable vehicles or carriers for the above noted formulations and compositions can be found in

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standard pharmaceutical texts, e.g. in "Remington's Pharmaceutical Sciences", The Science and Practice of Pharmacy, 19th Ed. Mack Publishing Company, Easton, Penn., (1995).

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Dosage levels of between about 0.01 and about 100 mg/kg body weight per day, preferably between about 0.5 and about 75 mg/kg body weight per day of the protease inhibitor compounds described herein are useful in a monotherapy for the prevention and 10 treatment of HCV mediated disease. Typically, the pharmaceutical compositions of this invention will be administered from about 1 to about 5 times per day or alternatively, as a continuous infusion. administration can be used as a chronic or acute 15 therapy. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. typical preparation will contain from about 5% to 20 about 95% active compound (w/w). Preferably, such preparations contain from about 20% to about 80% active compound.

As the skilled artisan will appreciate, lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the infection, the patient's disposition to the infection and the judgment of the treating

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physician. Generally, treatment is initiated with small dosages substantially less than the optimum dose of the peptide. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. In general, the compound is most desirably administered at a concentration level that will generally afford antivirally effective results without causing any harmful or deleterious side effects.

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When the compositions of this invention comprise a combination of a compound of formula I and one or more additional therapeutic or prophylactic agent, both the compound and the additional agent should be present at dosage levels of between about 10 to 100%, and more preferably between about 10 and 80% of the dosage normally administered in a monotherapy regimen.

20 When these compounds or their pharmaceutically acceptable salts are formulated together with a pharmaceutically acceptable carrier, the resulting composition may be administered in vivo to mammals, such as man, to inhibit HCV NS3 protease or to treat 25 or prevent HCV virus infection. Such treatment may also be achieved using the compounds of this invention in combination with agents which include, but are not limited to: immunomodulatory agents, such as α -, β -, or γ -interferons; other antiviral agents 30 such as ribavirin, amantadine; other inhibitors of HCV NS3 protease; inhibitors of other targets in the HCV life cycle such as helicase, polymerase, metalloprotease, or internal ribosome entry; or combinations thereof. The additional agents may be

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combined with the compounds of this invention to create a single dosage form. Alternatively these additional agents may be separately administered to a mammal as part of a multiple dosage form.

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Accordingly, another embodiment of this invention provides methods of inhibiting HVC NS3 protease activity in mammals by administering a compound of the formula I, wherein the substituents are as defined above.

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In a preferred embodiment, these methods are useful in decreasing HCV NS3 protease activity in a mammal. If the pharmaceutical composition comprises only a compound of this invention as the active component, such methods may additionally comprise the step of administering to said mammal an agent selected from an immunomodulatory agent, an antiviral agent, a HCV protease inhibitor, or an inhibitor of other targets in the HCV life cycle such as helicase, polymerase, or metallo protease. Such additional agent may be administered to the mammal prior to, concurrently with, or following the administration of the compositions of this invention.

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In an alternate preferred embodiment, these methods are useful for inhibiting viral replication in a mammal. Such methods are useful in treating or preventing HCV disease. If the pharmaceutical composition comprises only a compound of this invention as the active component, such methods may additionally comprise the step of administering to said mammal an agent selected from an immunomodulatory agent, an antiviral agent, a HCV

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protease inhibitor, or an inhibitor of other targets in the HCV life cycle. Such additional agent may be administered to the mammal prior to, concurrently with, or following the administration of the composition according to this invention.

The compounds set forth herein may also be used as laboratory reagents. The compounds of this invention may also be used to treat or prevent viral

10 contamination of materials and therefore reduce the risk of viral infection of laboratory or medical personnel or patients who come in contact with such materials (e.g. blood, tissue, surgical instruments and garments, laboratory instruments and garments,

15 and blood collection apparatuses and materials).

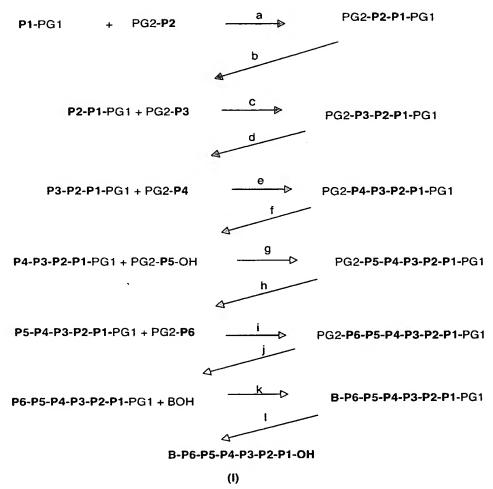
PROCESS

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The compounds of the present invention were synthesized according to the process as illustrated in scheme I (wherein PG1 is a carboxyl protecting group and PG2 is an amino protecting group):





Briefly, the P1, P2, P3, P4, and optionally P5 and P6 can be linked by well known peptide coupling techniques. The P1, P2, P3, P4, and P5 and P6 groups may be linked together in any order as long as the final compound corresponds to peptides of formula I. For example, P6 can be linked to P5 to give P5-P6 that is linked to P4-P3-P2-P1; or P6 linked to P5-P4-P3-P2 then linked to an appropriately C-terminal protected P1.

Generally, peptides are elongated by deprotecting the α -amino group of the N-terminal residue and coupling

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the unprotected carboxyl group of the next suitably N-protected amino acid through a peptide linkage using the methods described. This deprotection and coupling procedure is repeated until the desired 5 sequence is obtained. This coupling can be performed with the constituent amino acids in stepwise fashion, as depicted in Scheme I, or by condensation of fragments (two or several amino acids), or combination of both processes, or by solid phase peptide synthesis according to the method originally described in Merrifield, J. Am. Chem. Soc. (1963), 85, 2149-2154, the disclosure of which is hereby incorporated by reference.

15 Coupling between two amino acids, an amino acid and a peptide, or two peptide fragments can be carried out using standard coupling procedures such as the azide method, mixed carbonic-carboxylic acid anhydride (isobutyl chloroformate) method, carbodiimide 20 (dicyclohexylcarbodiimide, diisopropylcarbodiimide, or water-soluble carbodiimide) method, active ester (p-nitrophenyl ester, N-hydroxysuccinic imido ester) method, Woodward reagent K-method,

carbonyldiimidazole method, phosphorus reagents or oxidation-reduction methods. Some of these methods (especially the carbodiimide method) can be enhanced by adding 1-hydroxybenzotriazole. These coupling reactions can be performed in either solution (liquid phase) or solid phase.

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More explicitly, the coupling step involves the dehydrative coupling of a free carboxyl of one reactant with the free amino group of the other reactant in the presence of a coupling agent to form

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a linking amide bond. Descriptions of such coupling agents are found in general textbooks on peptide chemistry, for example, M. Bodanszky, "Peptide Chemistry", 2nd rev ed., Springer-Verlag, Berlin, Germany, (1993). Examples of suitable coupling agents are N, N'-dicyclohexylcarbodiimide, hydroxybenzotriazole in the presence of N, N'dicyclohexylcarbodiimide or N-ethyl-N'-[(3dimethylamino)propyl]carbodiimide. A very practical and useful coupling agent is the commercially available (benzotriazol-1-yloxy)tris-(dimethylamino) phosphonium hexafluorophosphate, either by itself or in the presence of 1hydroxybenzotriazole. Another very practical and useful coupling agent is commercially available 2-(1H-benzotriazol-1-yl)-N, N, N', N'tetramethyluronium tetrafluoroborate. Still another very practical and useful coupling agent is commercially available O-(7-azabenzotriazol-1-yl)-N, N, N', N'-tetramethyluronium hexafluorophosphate.

The coupling reaction is conducted in an inert solvent, e.g. dichloromethane, acetonitrile or dimethylformamide. An excess of a tertiary amine, e.g. diisopropylethylamine, N-methylmorpholine or N-methylpyrrolidine, is added to maintain the reaction mixture at a pH of about 8. The reaction temperature usually ranges between 0°C and 50°C and the reaction time usually ranges between 15 min and 24 h.

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When a solid phase synthetic approach is employed, the C-terminal carboxylic acid is attached to an insoluble carrier (usually polystyrene). These insoluble carriers contain a group that will react

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with the carboxylic group to form a bond that is stable to the elongation conditions but readily cleaved later. Examples of which are: chloro- or bromomethyl resin, hydroxymethyl resin, and aminomethyl resin. Many of these resins are commercially available with the desired C-terminal amino acid already incorporated. Alternatively, the amino acid can be incorporated on the solid support by known methods Wang, S.-S., J. Am. Chem. Soc., (1973), 95, 1328; Atherton, E.; Shepard, R.C. "Solidphase peptide synthesis; a practical approach" IRL Press: Oxford, (1989); 131-148. In addition to the foregoing, other methods of peptide synthesis are described in Stewart and Young, "Solid Phase Peptide Synthesis", 2nd ed., Pierce Chemical Co., Rockford, IL (1984); Gross, Meienhofer, Udenfriend, Eds., "The Peptides: Analysis, Synthesis, Biology", Vol. 1, 2, 3, 5, and 9, Academic Press, New-York, (1980-1987); Bodansky et al., "The Practice of Peptide Synthesis" Springer-Verlag, New-York (1984), the disclosures of

The functional groups of the constituent amino acids generally must be protected during the coupling

25 reactions to avoid formation of undesired bonds. The protecting groups that can be used are listed in Greene, "Protective Groups in Organic Chemistry",

John Wiley & Sons, New York (1981) and "The Peptides: Analysis, Synthesis, Biology", Vol. 3, Academic

Press, New York (1981), the disclosures of which are hereby incorporated by reference.

which are hereby incorporated by reference.

The α -carboxyl group of the C-terminal residue is usually protected as an ester (PG1) that can be

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cleaved to give the carboxylic acid. Protecting groups that can be used include: 1) alkyl esters such as methyl, trimethylsilylethyl and t-butyl, 2) aralkyl esters such as benzyl and substituted benzyl, or 3) esters that can be cleaved by mild base treatment or mild reductive means such as trichloroethyl and phenacyl esters.

The α -amino group of each amino acid to be coupled to 10 the growing peptide chain must be protected (PG2). Any protecting group known in the art can be used. Examples of such groups include: 1) acyl groups such as formyl, trifluoroacetyl, phthalyl, and ptoluenesulfonyl; 2) aromatic carbamate groups such as 15 benzyloxycarbonyl (Cbz or Z) and substituted benzyloxycarbonyls, and 9-fluorenylmethyloxycarbonyl (Fmoc); 3) aliphatic carbamate groups such as tertbutyloxycarbonyl (Boc), ethoxycarbonyl, diisopropylmethoxycarbonyl, and allyloxycarbonyl; 4) 20 cyclic alkyl carbamate groups such as cyclopentyloxycarbonyl and adamantyloxycarbonyl; 5) alkyl groups such as triphenylmethyl and benzyl; 6) trialkylsilyl such as trimethylsilyl; and 7) thiol containing groups such as phenylthiocarbonyl and 25 dithiasuccinoyl. The preferred α -amino protecting group is either Boc or Fmoc. Many amino acid derivatives suitably protected for peptide synthesis are commercially available.

The α-amino protecting group of the newly added amino acid residue is cleaved prior to the coupling of the next amino acid. When the Boc group is used, the methods of choice are trifluoroacetic acid, neat or in dichloromethane, or HCl in dioxane or in ethyl

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acetate. The resulting ammonium salt is then neutralized either prior to the coupling or in situ with basic solutions such as aqueous buffers, or tertiary amines in dichloromethane or acetonitrile or dimethylformamide. When the Fmoc group is used, the reagents of choice are piperidine or substituted piperidine in dimethylformamide, but any secondary amine can be used. The deprotection is carried out at a temperature between 0°C and room temperature (RT), usually 20-22°C.

Any of the amino acids having side chain functionalities must be protected during the preparation of the peptide using any of the above-described groups. Those skilled in the art will appreciate that the selection and use of appropriate protecting groups for these side chain functionalities depend upon the amino acid and presence of other protecting groups in the peptide. The selection of such protecting groups is important in that the group must not be removed during the deprotection and coupling of the α -amino group.

For example, when Boc is used as the α-amino

25 protecting group, the following side chain protecting
groups are suitable: p-toluenesulfonyl (tosyl)

moieties can be used to protect the amino side chain
of amino acids such as Lys and Arg; acetamidomethyl,
benzyl (Bn), or t-butylsulfonyl moieties can be used

to protect the sulfide containing side chain of
cysteine; benzyl (Bn) ethers can be used to protect
the hydroxy containing side chains of serine,
threonine or hydroxyproline; and benzyl esters can be

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used to protect the carboxy containing side chains of aspartic acid and glutamic acid.

When Fmoc is chosen for the α-amine protection,
usually tert-butyl based protecting groups are acceptable. For instance, Boc can be used for lysine and arginine, tert-butyl ether for serine, threonine and hydroxyproline, and tert-butyl ester for aspartic acid and glutamic acid. Triphenylmethyl (Trityl)
moiety can be used to protect the sulfide containing side chain of cysteine.

Once the elongation of the peptide is completed all of the protecting groups are removed. When a liquid phase synthesis is used, the protecting groups are removed in whatever manner is dictated by the choice of protecting groups. These procedures are well known to those skilled in the art.

20 When a solid phase synthesis is used, the peptide is cleaved from the resin simultaneously with the removal of the protecting groups. When the Boc protection method is used in the synthesis, treatment with anhydrous HF containing additives such as 25 dimethyl sulfide, anisole, thioanisole, or p-cresol at 0°C is the preferred method for cleaving the peptide from the resin. The cleavage of the peptide can also be accomplished by other acid reagents such as trifluoromethanesulfonic acid/ trifluoroacetic 30 acid mixtures. If the Fmoc protection method is used, the N-terminal Fmoc group is cleaved with reagents described earlier. The other protecting groups and the peptide are cleaved from the resin

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using solution of trifluoroacetic acid and various additives such as anisole, etc.

When **Q** is CH₂, **a** is 0, **b** is 0 and **B** is **R**_{11a}N(**R**_{11b})C(O), the compounds were prepared according to a method analogous to the general method described for the peptides in Scheme I using a readily available succinyl intermediate, t-BuO-C(O)CH₂CH(**R**₄)-CO-PG1 (e.g. PG1= 2-oxo-4-substituted-oxazolidin-3-yl).

- This succinyl intermediate can easily be prepared according to the method of Evans'et al (J. Am. Chem. Soc. (1982), 104, 1737) using the appropriate 4-substituted-3-acyl-2-oxazolidinone in the presence of a strong base such as lithium diisopropylamide or
- sodium bis(trimethylsilyl)amide and t-butyl bromoacetate. After cleavage of the 2-oxazolidinone moiety with LiOOH (Evans'et al., Tetrahedron Lett. (1987), 28, 6141), the resulting acid was coupled to the P3-P2-P1-PG1 segment to give t-BuO-C(O)-
- 20 $CH_2CH(R_4)-CO-P3-P2-P1-PG1$. The latter was treated with hydrogen chloride to selectively convert the terminal t-butyl ester into the corresponding acid that was finally coupled to $R_{11a}NH(R_{11b})$ to give, after removal of the protective group(s), the desired
- peptide derivative. The amines R_{11a}NH(R_{11b}) are commercially available or the synthesis is well known in the art. A specific embodiment of this process is presented in Example 18.
- 30 Alternatively, starting with the same succinyl intermediate (t-BuO-C(O)CH₂CH(R₄)-CO-PG1), the sequence of reactions can be inverted to introduce first $\mathbf{R_{11a}}$ NH($\mathbf{R_{11b}}$) and then P3-P2-P1-PG1 to give the desired peptide derivative.

Synthesis of capping group B and P6, P5, P4, and P3 moieties

Different capping groups **B** are introduced to

5 protected P6, P5, P4, the whole peptide or to any
peptide segment with an appropriate acyl chloride
that is either available commercially or for which
the synthesis is well known in the art.

Different **P6** to **P3** moieties are available commercially or the synthesis is well known in the art.

Synthesis of P2 moieties.

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1. Synthesis of precursors:

A) Synthesis of haloarylmethane derivatives.

The preparation of halomethyl-8-quinoline **IId** was done according to the procedure of K.N. Campbell et al., J. Amer. Chem. Soc., (1946), 68, 1844.

Scheme II

Briefly, 8-quinoline carboxylic acid **IIa** was converted to the corresponding alcohol **IIc** by reduction of the corresponding acyl halide **IIb** with a reducing agent such as lithium aluminium hydride. Treatment of alcohol **IIb** with the appropriate hydrohaloacid gives the desired halo derivative **IId.** A specific embodiments of this process is presented in Example 1.

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2. Synthesis of P2:

A) The synthesis of 4-substituted proline (wherein \mathbb{R}^2 is attached to the ring via a carbon atom) (with the stereochemistry as shown):

is done as shown in Scheme III according to the procedures described by J. Ezquerra et al. (Tetrahedron, (1993), 38, 8665-8678) and C. Pedregal et al. (Tetrahedron Lett., (1994), 35, 2053-2056).

Scheme III

Briefly, Boc-pyroglutamic acid is protected as a benzyl ester. Treatment with a strong base such as lithium diisopropylamide followed by addition of an alkylating agent $(Br-R^2 \text{ or } I-R^2)$ gives the desired compounds **IIIe** after reduction of the amide and deprotection of the ester.

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B) The synthesis of O-alkylated 4-(R)-hydroxyproline:

may be carried out using the different processes described below.

B.1) When R¹² is aralkyl, the process can be carried out according to the procedure described by E.M. Smith et al. (J. Med. Chem. (1988), 31, 875-885). Briefly, commercially available Boc-4(R)-hydroxyproline is treated with a base such as sodium hydride and the resulting alkoxide reacted with an alkylating agent (Br-R¹² or I-R¹²) to give the desired compounds. Specific embodiments of this process are presented in Examples 3 and 4.

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B.2) When \mathbf{R}^{12} is aryl, the compounds can be prepared via a Mitsunobu reaction (Mitsunobu (1981), Synthesis, January, 1-28; Rano et al., (1995), Tet. Lett. <u>36(22)</u>, 3779-3792; Krchnak *et* 20 al., (1995), Tet. Lett. 36(5), 62193-6196; Richter et al., (1994), Tet. Lett. 35(27), 4705-4706). Briefly, commercially available Boc-4(S)-hydroxyproline methyl ester is treated with the appropriate aryl alcohol or thiol in the 25 presence of triphenylphosphine and diethylazodicarboxylate (DEAD) and the resulting ester is hydrolysed to the acid. Specific embodiments of this process are presented in Examples 5 and 6.

PCT/CA98/00765

Scheme IV

Alternatively, the Mitsunobu reaction can be produced in solid phase (as shown in Scheme IV). The 96-well block of the Model 396 synthesizer (advanced ChemTech) is provided with aliquots of resin-bound compound (IVa) and a variety of aryl alcohols or thiols and appropriate reagents are added. After incubation, each resin-bound product (IVb) is washed, dried, and cleaved from the resin.

B.2.a) A Suzuki reaction (Miyaura et al., (1981), Synth. Comm. 11, 513; Sato et al., (1989), Chem. Lett., 1405; Watanabe et al., (1992), Synlett., 207; Takayuki et al., (1993), J. Org. Chem. 58, 2201; Frenette et al., (1994), Tet. Lett. 35(49), 9177-9180; Guiles et al., (1996), J. Org. Chem. 61, 5169-5171) can also be used to further functionalize the aryl substituent.

Examples

The present invention is illustrated in further detail by the following non-limiting examples.

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Temperatures are given in degrees Celsius. Solution percentages express a weight to volume relationship, and solution ratios express a volume to volume relationship, unless stated otherwise. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 MHz spectrometer; the chemical shifts (δ) are reported in parts per million. Flash chromatography was carried out on silica gel (SiO₂)

according to Still's flash chromatography technique (W.C. Still et al., J. Org. Chem. (1978), 43, 2923).

Abbreviations used in the examples include Bn:
benzyl; Boc: tert-butyloxycarbonyl {Me₃COC(0)}; BSA:
bovine serum albumin; CHAPS: 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; DBU: 1,8diazabicyclo[5.4.0]undec-7-ene; CH₂Cl₂= DCM:
methylene chloride; DIPEA: diisopropylethylamine;
DMAP: dimethylaminopyridine; DCC: 1,3dicyclohexylcarbodiimide; DME: 1,2-dimethyoxyethane;
DMF: dimethylformamide; DMSO: dimethylsulfoxide; DTT:
dithiothreitol or threo-1,4-dimercapto-2,3butanediol; EDTA: ethylenediaminetetraacetic acid;
Et: ethyl; EtOH: ethanol; EtOAc: ethyl acetate; Et₂O:
diethyl ether; HPLC: high performance liquid

chromatography; MS: mass spectrometry (MALDI-TOF:
Matrix Assisted Laser Disorption Ionisation-Time of
Flight, FAB: Fast Atom Bombardment); LAH: lithium
aluminum hydride; Me: methyl; MeOH: methanol; MES:
(2-{N-morpholino}ethane-sulfonic acid); NaHMDS:

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sodium bis(trimethylsilyl)amide; NMM: Nmethylmorpholine; NMP: N-methylpyrrolidine; Pr: propyl; Succ: 4-hydroxy-1,4-dioxobutyl; PNA: 4nitrophenylamino or p-nitroanalide; TBAF: tetra-n-5 butylammonium fluoride; TCEP: tris(2-carboxyethyl) phosphine hydrochloride; TFA: trifluoroacetic acid; THF: tetrahydrofuran; TIS: triisopropylsilane; TLC: thin layer chromatography; TMSE: trimethylsilylethyl; Tris/HCl: tris(hydroxymethyl)aminomethane hydrochloride.

Example 1

Synthesis of bromomethyl-8-quinoline (1):

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To commercially available 8-quinoline carboxylic acid (2.5 g, 14.4 mmol) was added neat thionyl chloride 20 (10 ml, 144 mmol). This mixture was heated at 80° C for 1 h before the excess thionyl chloride was distilled off under reduced pressure. To the resulting brownish solid was added absolute EtOH (15 mL) which was heated at 80°C for 1 h before being 25 concentrated in vacuo. The residue was partitioned between EtOAc and saturated aqueous NaHCO3, and the organic phase dried (MgSO₄), filtered and concentrated to give a brownish oil (2.8 g). This material (ca. 14.4 mmol) was added dropwise over 35 30 min to a LAH (0.76 g, 20.2 mmol)/Et₂O suspension which was cooled to -60°C. The reaction mixture was slowly warmed to -35°C over 1.5 h before the reaction

was complete. The reaction was quenched with MgSO₄.10H₂O slowly over 30 min and then wet THF. The mixture was partitioned between Et₂O and 10% aqueous NaHCO₃. The organic phase was dried (MgSO₄), filtered and concentrated to give a yellowish solid (2.31 g, 80% over 2 steps) corresponding to the alcohol. The alcohol (2.3 g, 11.44 mmol) was dissolved in AcOH/HBr (20 mL, 30% solution from Aldrich) and heated at 70°C for 2.5 h. The mixture was concentrated *in vacuo* to dryness, partitioned between EtOAc (100 mL) and saturated aqueous NaHCO₃ before being dried (MgSO₄), filtered and concentrated to give the desired compound (1) as a brownish solid (2.54 g, 100%).

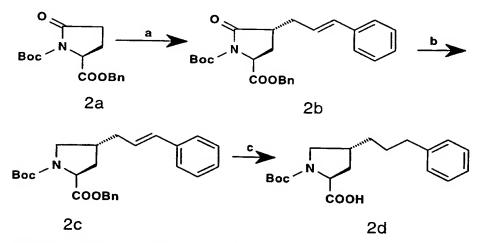
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Example 2

Synthesis of Boc-4(R)-(3-phenylpropyl)proline (2d).



20 a) Synthesis of compound 2b:

To a solution of Boc-pyroglutamic acid benzyl ester (2a) (prepared as described by A.L Johnson et al., J. Med. Chem. (1985), 28, 1596-1602) (500 mg, 1.57 mmol) in THF (10 mL) at -78 °C, was slowly added lithium hexamethydisilylazide (1.72 mL, 1M solution in THF).

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After stirring for 1 h at -78°C, cinnamyl bromide (278 μ L, 1.88 mmol) was added and the stirring continued for an additional 2 h. The reaction mixture was quenched with saturated ammonium chloride 5 solution and extracted with ethyl ether $(3 \times 20 \text{ mL})$. The combined organic extracts were dried (MgSO₄), filtered and concentrated. The residue was purified by flash column chromatography (8:2 hexane:ethyl acetate) to give compound 2b as an off-white solid (367 mg, 54% yield). ¹H NMR (CDCl₃): δ 7.35-7.19 (m, 10 10H), 6.43 (d, J=15 Hz, 1H), 6.11 (ddd, J=15, J'=J''=8Hz, 1 H), 5.26 (d, J=16 Hz, 1H), 5.17 (d, J=16 Hz, 1H), 4.59 (dd, J=9.5, J'=2 Hz, 1 H), 2.83-2.70 (m, 2H), 2.41-2.34 (m, 1H), 2.22-2.16 (m, 1H), 2.10-2.02 15

b) Synthesis of compound 2c:

(m, 1H) 1.42 (s, 9 H).

At -78°C, lithium triethylborohydride (1M solution in THF, 1.01 mL, 1.01 mmol) was added to a solution of 20 compound 2b (367 mg, 0.843 mmol) in THF (5 mL), under a nitrogen atmosphere. After 30 min, the reaction mixture was quenched with saturated aqueous NaHCO3 (2 mL) and warmed to 0° C. 30% H_2O_2 (5 drops) was added and the mixture was stirred at 0°C for 20 min. 25 organic volatiles were removed in vacuo, and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic extracts were dried (MgSO₄), filtered and concentrated. To a cold (-78°C) solution of the residue and triethylsilane (134 µL, 0.843 mmol) in CH_2Cl_2 (3 mL) boron trifluoride 30 etherate (118 μ L,0.927 mmol) was added dropwise under an atmosphere of nitrogen. After 30 min, additional triethylsilane (134 μ L) and boron trifluoride

etherate (118 μL) were added. After stirring for 2 h at -78°C , the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (2 mL) and extracted with DCM (3 \times 10 mL). The combined organic extracts were dried $(MgSO_4)$, filtered and concentrated. The crude 5 product was purified by flash column chromatography (8:2 hexane:ethyl acetate) to give compound 2c as a colorless oil (140 mg, 40% yield). ^{1}H NMR (CDCl $_{3}$) indicated the presence of two rotamers: $\delta \ 7.34\text{--}7.22$ (m, 10H), 6.38 (d, J=15.5 Hz, 1H), 6.15-6.08 (m, 1H), 10 5.29-5.07 (m, 2H), 4.44 (d, J=7 Hz, 1/3H), 4.33 (d, J=7 Hz, 2/3H), 3.76 (dd, J=10.5, J'=8.5 Hz, <math>2/3H), 3.69 (dd, J=10.5, J'=8.5 Hz, 1/3H), 3.13 (dd, J=9, J'=8.5 Hz, 2/3H), 3.05 (dd, J=9, J'=8.5 Hz, <math>1/3H), 2.47-2.40 (m, 1H), 2.35-2.22 (m, 2H) 2.15-1.85 (m, 15 2H), 1.45 (s, (3/9) 9H), 1.33 (s, (6/9) 9H).

c) Synthesis of compound 2d:

To a solution of compound 20 (140 mg, 0.332 mmol) in ethanol (4 mL) was added 10% palladium on charcoal 20 (30 mg). The mixture was stirred under an atmosphere of hydrogen for 2 h. The catalyst was removed by passing the mixture through a Millipore: Millex - HV $0.45~\mu\text{m}$ filter. The clear solution was concentrated to give the desired compound 2d as a colorless oil 25 (115 mg, quant. yield). ^{1}H NMR (DMSO-d₆) indicated the presence of two rotamers: δ 7.28-7.14 (m, 5H), 4.33 (br.s, 1H), 4.06-4.10, (m, 1H), 3.56-3.42 (m, 3H), 2.89-2.79 (m, 1H),), 2.53-2.49 (m, 1H, under DMSO- d_6), 2.24-2.10 (m, 1H), 2.03-1.93 (m, 1H), 1.87-30 1.75 (m, 1H), 1.62-1.45 (m, 2H), 1.38 (s, (3/9) 9H),1.33 (s, (6/9) 9H).

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Example 3

Synthesis of Boc-4(R)-(naphthalen-1-ylmethoxy)

proline (3):

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Commercially available Boc-4(R)-hydroxyproline (5.00 g, 21.6 mmol) was dissolved in THF (100 mL) and cooled to 0°C. Sodium hydride (60% dispersion in oil, 10 1.85 g, 45.4 mmol) was added portionwise over 10 minutes and the suspension was stirred at RT for 1 h. Then, 1-(bromomethyl)naphthalene (8.00 g, 36.2 mmol) (prepared as described in E.A. Dixon et al. Can. J. Chem., (1981), 59, 2629-2641) was added and the 15 mixture was heated at reflux for 18 h. The mixture was poured into water (300 mL) and washed with hexane. The aqueous layer was acidified with 10% aqueous HCl and extracted twice with ethyl acetate. The organic layers were combined and washed with 20 brine, dried (MgSO₄), filtered and concentrated. residue was purified by flash chromatography (49:49:2 hexane: ethyl acetate: acetic acid) to give the title compound as a colorless oil (4.51 g, 56% yield). NMR (DMSO-d₆) indicated the presence of two rotamers: 25 δ 8.05 (m, 1H), 7.94 (m, 1H), 7.29 (d, J=14 Hz, 1H), 7.55-7.45 (m, 4H), 4.96 (m, 2H), 4.26 (br. s, 1H), 4.12 (dd, J=J=8 Hz, 1H), 3.54-3.42 (m, 2H), 2.45-2.34 (m, 1H), 2.07-1.98 (m, 1H) 1.36 (s, (3/9) 9H), 1.34 (s, (6/9) 9H).

Example 4

Synthesis of Boc-4(R)-(8-quinoline-methyloxy) proline (4):

Boc-4(R)-hydroxyproline (1.96 g, 8.5 mmol) in 5 anhydrous THF (20 mL) was added to a suspension of NaH (1.4 g, 60% in oil, 34 mmol) in THF (100 mL). This mixture was stirred 30 min before bromomethyl-8quinoline from Example 1 (2.54 g, 11.44 mmol) was 10 added in THF (30 mL). The reaction mixture was heated at 70°C (5 h) before the excess NaH was destroyed carefully with wet THF. The reaction was concentrated in vacuo and the resulting material was dissolved in EtOAc and H_2O . The basic aqueous phase was separated 15 and acidified with 10% aqueous HCl to pH ~5 before being extracted with EtOAc (150 mL). The organic phase was dried $(MgSO_4)$, filtered and concentrated to give a brown oil. Purification by flash chromatography (eluent: 10% MeOH/CHCl3) gave the 20 desired compound as a pale yellow solid (2.73 g, 86%). HPLC (97.5%); 1H-NMR (DMSO- d_6) shows rotamer populations in a 6:4 ratio, δ 12-11.4 (bs, 1H), 8.92 $(2 \times d, J = 4.14 \text{ and } 4.14 \text{ Hz}, 1\text{H}), 8.38 (2 \times d, J =$ 8.27 and 8.27 Hz, 1H), 7.91 (d, J = 7.94 Hz, 1H), 25 7.77 (d, J = 7.0 Hz, 1H), 7.63-7.54 (m, 2H), 5.14 (2 \times s, 2H), 4.32-4.29 (m, 1H), 4.14-4.07 (m, 1H), 3.52-3.44 (m, 2H), 2.43-2.27 (m, 1H), 2.13-2.04 (m, 1H),

1.36 and 1.34 (2 x s, 9H).

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Example 5

Preparation of Boc-4(R)-(7-chloroquinoline-4-oxo)proline (5):

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Commercially available Boc-4(S)-hydroxyproline methyl ester (500 mg, 2.04 mmol) and 7-chloro-4hydroxyquinoline (440 mg, 2.45 mmol) were placed in dry THF (10 mL) at 0°C. Triphenylphosphine (641 mg, 2.95 mmol) was added, followed by slow addition of 10 DIAD (426 mg, 2.45 mmol). The mixture was stirred at RT for 20 h. The reaction mixture was then concentrated, taken up in ethyl acetate and extracted three times with HCl 1N. The aqueous phase was 15 basified with Na₂CO₃ and extracted twice with ethyl acetate. The organic layers were combined, dried over MgSO₄, filtered and concentrated to give a yellow oil. The oil was purified by flash chromatography to give compound (5) methyl ester as 20 a white solid, 498 mg, 58% yield.

This methyl ester (400 mg, 0.986 mmol) was hydrolysed with 1M aqueous sodium hydroxide (1.7 mL, 1.7 mmol) in methanol (4 mL), at 0°C, for 3 h. The solution was concentrated to remove the methanol and neutralised with 1M aqueous HCl. The suspension was concentrated to dryness and taken up in methanol (20 mL), the salts were filtered off and the filtrate concentrated to give the desired compound (5) as a

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white solid, 387 mg, quant. yield.

¹H NMR (DMSO-d₆) (ca. 1:1 mixture of rotamers) δ 8.74 (d, J = 5 Hz, 1 H), 8.13-8.09 (m, 1 H), 7.99 and 7.98 (s, 1 H), 7.58 (d, J = 9 Hz, 1 H), 7.02 (d, J = 5 Hz, 1 H), 5.26-5.20 (m, 1 H), 4.10-4.01 (m, 1 H), 3.81-3.72 (m, 1 H), 3.59 (dd, J = 12, 10 Hz, 1 H), 2.41-2.31 (m, 2 H), 1.34 and 1.31 (s, 9H).

Example 6

General procedure for Mitsunobu reaction in solid phase (Scheme IV)

The polymer-bound peptide of general structure IVa (0.327 mmoles of peptide per gram of Wang resin) was dried under high vacuum in a desiccator over P_2O_5 .

- The 96-well block of the Advanced ChemTech Model 396 synthesizer was furnished with aliquots of IVa (120 mg, 0.04 mmol peptide per well) and each sample was washed for 5 min with anhydrous CH_2Cl_2 (5x1200 μ L) and then with anhydrous THF (5x1500 μ L). Anhydrous
- THF (200 μ L) was added to each sample and the synthesizer was temporarily stopped to allow the manual addition of reagents. Ph $_3$ P (5 eq. in 400 μ L of anhydrous THF) and diethylazodicarboxylate (DIAD, 5 eq. in 250 μ L of anhydrous THF)) were added to
- each sample before the addition of a phenol or thiophenol reagent (5 eq, 0.2 mmol, dissolved in 500 μL of anhydrous THF); a library of reagents was used to produce the library of HCV protease inhibitors described in this patent application. After the
- addition of all reagents, the mixtures were shaken for a total of 4 h with a 10 min delay after each hour. Each resin-bound product was washed with THF (2x1500 μ L), DMF (4x1500 μ L), isopropanol (4x1500

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 μ L), CH₂Cl₂ (4x1500 μ L) and finally methanol (2x1500 μ L). The sample was dried under vacuum and then treated with 40% TFA in CH₂Cl₂ for 1 h in order to cleave the peptide product (general structure **IVb**) from the resin. All products were purified by preparative HPLC on a reversed phase C18 column using a linear solvent gradient from 5% aqueous CH₃CN to 100% CH₃CN.

The following description is an example of the further elaboration of the side chain R₁₂ at P2 by the application of a biaryl synthesis via Suzuki coupling on a solid support (cf. R. Frenette and R.W. Friesen, Tetrahedron Lett. (1994), 35, 9177).

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The precursor, aromatic bromide compound 238 of Table 2, was first synthesized from the polymer-bound tetrapeptide having a *cis*-hydroxyproline at the P2 position and 4-bromophenol using the Mitsunobu protocol described above.

Example 7

Suzuki Library of Reactions in Solid Phase Synthesis

All reactions were carried out in 16x100 mm, high pressure screw-cap test tubes with teflon caps, equipped with small magnetic stirring bars. For each reaction, a degassed suspension of the polymer-bound peptide (100 mg of Wang resin with 0.033 mmol of bound peptide) was first added to the test tube, followed by the addition of DME (2 mL), Pd(Ph₃P)₃ (~3 mg, 0.05 eq.), Na₂CO₃ (70 μL of a 2M solution in H₂O, 2.5 eq.) and one of the phenyl boronic acid reagents from our library. The test tubes were flashed with

nitrogen gas, sealed and placed in an oil bath at 80°C. All of the reactions were stirred gently and allowed to proceed for 15-18 h. Each resin bound peptide product was subsequently transferred into a plastic filtration tube, washed with DME:H₂O (1:1, 5x 2 mL), DME (5x 2 mL), methanol (5x 2 mL), CH₃CN (5x 2 mL), CH₂Cl₂ (5x 2 mL) and dried under high vacuum. Each product was cleaved from the resin by treating the sample with 45% TFA in CH₂Cl₂ (1 mL) for 1 hour. All products were purified by preparative HPLC on a reversed phase C18 column using a solvent linear gradient from 5% aqueous CH₃CN to 100% CH₃CN.

Example 8

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Preparation of a library of Ac-Chg-Val-Hyp(aryl)-Acca-OH

This compound was synthesized in accordance with the protocol of Example 6 where appropriate peptides were used.

Example 9

Synthesis of Polymer-Bound Compound #246 of Table 2.

The synthesis of compound **246** was done according to the process Example 7.

Compound 246:

ES $^{-}$ MS m/z 675.3 [(M-H) $^{-}$]; ~95% pure by C18 reversed phase HPLC; Mixture of two rotamers in a ratio of ~1:3 based on 1 H NMR

- ¹H NMR of major rotamer (400 MHz, DMSO): δ 8.44 (s, 1H), 7.84 (d, J=8.6 Hz, 1H), 7.82 (d, J=~8.6 Hz, 1H), 7.54 (bd, J=8.3 Hz, 4H), 6.99 (d, J=8.9 Hz, 2H), 6.98 (d, J=8.9 Hz, 2H), 5.11 (bs, 1H), 4.29-4.34 (m, 2H), 4.21 (bt, J=7.8 Hz, 1H), 3.94-4.02 (m, 2H), 3.78 (s,
- 10 3H), 2.29-2.33 (m, 2H), 2.15-2.21 (m, 1H), 1.95-1.99 (m, 1H), 1.83 (s, 3H), 1.45-1.70 (m, 8H), 1.33-1.40 (m, 1H), 1.20-1.28 (m, 1H), 1.02-1.18 (m, 2H), ~0.9-1.02 (m, 2H), 0.90 (d, J= 6.7 Hz, 3H) 0.84 (d, J=6.7 Hz, 3H).

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Example 10

General procedure for coupling reactions done in solution (See also R. Knorr et al., Tetrahedron Letters, 30, 1927 (1989).)

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The reactants, i.e. a free amine (1 eq.) (or its hydrochloride salt) and the free carboxylic acid (1 eq.) were dissolved in CH_2Cl_2 , CH_3CN or DMF. Under a nitrogen atmosphere, four equivalents of N-

- 25 methylmorpholine and 1.05 equivalents of the coupling agent were added to the stirred solution. After 20 min, one equivalent of the second reactant, i.e. a free carboxylic acid was added. (Practical and efficient coupling reagents for this purpose are
- 30 (benzotriazol-1-yloxy) tris-(dimethylamino) phosphonium
 hexafluorophosphate (HOBT) or preferably 2-(1Hbenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium
 tetrafluoroborate (TBTU) or 0-(7-azabenzotriazol-1yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate

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(HATU). The reaction was monitored by TLC. After completion of the reaction, the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc. The solution was washed successively with 10% aqueous citric acid, saturated aqueous NaHCO3 and brine. The organic phase was dried (MgSO4), filtered and concentrated under reduced pressure. When the residue was purified, it was done by flash chromatography as defined above.

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Example 11

Synthesis of "tripeptide segment": Ac-Chg-Chg-Pro (4(R)-naphthalen-1-ylmethoxy)-OH (11g)

11a

11b

Compound 11a (4.45g , 11.98 mmol) was dissolved in anhydrous CH₃CN (60 mL). DBU (2.2 mL , 14.38mmol) and allyl bromide (1.1mL , 13.18 mmol) were added 5 successively and the reaction mixture was stirred 24 h at RT The mixture was concentrated, the resulting oil was diluted with EtOAc and water and successively washed with water (2x) and brine (1x). The EtOAc layer was dried (MgSO₄), filtered and evaporated to 10 dryness. The yellow oil was purified by flash chromatography (eluent:hexane:EtOAc;90:10 to 85:15) to provide the product 11b as a yellow oil (2, 4.17g ; 85 % yield). MS (FAB) 412 MH+ 1 H NMR (CDCl $_{3}$) , mixture of rotamers ca.1:2 , δ (d, J= 15 8Hz, 1H), 7.87 (d, J=8Hz, 1H), 7.82 (d, J=8Hz, 1H), 7.55-7.41 (m, 4H), 5.95-5.85 (m, 1H), 5.34-5.21 (m, 2H), 5.03-4.88 (m, 2H), 4.70-4.56 (m, 2H), 4.48 & 4.39 (t, J=8, 15Hz, 1H), 4.28-4.23 (m, 1H), 3.81-3.55 (m, 2H), 2.46-2.36 (m, 1H), 2.13-2.05 (m, 1H), 20 1.44 &1.41 (s, 9H).

Compound 11b (2.08 g , 5.05 mmol) was treated for 30 min at RT with 4N HCl / dioxane. Evaporation to dryness provided the corresponding amine-HCl as an oil. The amine-HCl 11c was dissolved in anhydrous DCM (25 mL), NMM (2.2 mL, 20.22 mmol), Boc-Chg-OH · H₂O (1.53 g, 5.56 mmol) and TBTU (1.95 g, 6.07 mmol) were added successively. The reaction mixture was stirred at RT overnight, then, diluted with EtOAc and

successively washed with 10% aqueous citric acid (2x), saturated aq. NaHCO₃ (2x), water (2x), and brine (1x). The EtOAc layer was dried (MgSO₄), filtered and evaporated to dryness to provide the crude product 11d as a yellowish-white foam (ca 2.78 5 g, 100% yield). MS (FAB) 551.4 MH^+ . 1H NMR (CDCl₃) δ 8.03(d, J= 8Hz, 1H), 7.86 (b d, J= 8.5Hz, 1H), 7.84 (d, J=8Hz, 1H), 7.56-7.40 (m, 4H), 5.92-5.85 (m, 1H), 5.31 (dd, J= 1, 17Hz, 1H), 5.22 (dd, J= 1, 10Hz, 10 1H), 5.17 (d, J=9Hz, 1H), 5.05 (d, J=12Hz, 1H), 4.91 (d, J= 12Hz, 1H), 4.67-4.60 (m, 3H), 4.31-4.27 (m, 2H), 4.16 (b d, J= 11Hz, 1H), 3.71 (dd, J= 4, 11Hz, 1H), 2.47-2.41 (m, 1H), 2.08-1.99 (m,1H), 1.85-1.63 (m, 5H), 1.44-1.40 (m, 1H), 1.36 (s, 9H), 1.28-15 1.00 (m, 5H).

The crude dipeptide 11d (ca. 5.05 mmol) was treated with 4N HCl/dioxane (25 mL) as described for compound 11c. The crude hydrochloride salt was coupled to Boc-Chg-OH H₂O (1.53g, 5.55 mmol) with NMM (2.22 mL, 20.22 mmol) and TBTU (1.95 g, 6.07 mmol) in DCM (25 mL) as described for compound 11d to yield crude tripeptide as a yellow-oil foam. The crude material was purified by flash chromatography

(eluent:hexane:EtOAc;80:20 to 75:25) to provide the

tripeptide **11e** as a white foam (2.75g; 79% yield over 2 steps). MS (FAB) 690.5 MH⁺. ¹H NMR (CDCl₃), mainly one rotamer, δ 8.06 (d, J= 8Hz, 1H), 7.87 (b d, J= 8.5Hz, 1H), 7.82 (d, J= 8Hz, 1H), 7.57-7.40 (m, 4H), 6.41 (d, J= 8.5Hz, 1H), 5.92-5.84 (m, 1H), 5.31 (dd, J= 1, 17Hz, 1H), 5.23 (dd, J= 1, 10.5Hz, 1H), 5.04 (d, J= 12Hz, 1H), 4.98 (b d, J= 7Hz, 1H), 4.93 (d, J=12Hz, 1H), 4.63-4.58 (m, 4H), 4.29-4.25 (m, 1H), 4.10-4.07 (m, 1H), 3.90-3.84 (m, 1H), 3.72 (dd,

71 J= 4, 11Hz, 1H), 2.48-2.40 (m, 1H), 2.07-1.99 (m, 1H), 1.83-1.55 (m, 12H), 1.43 (s, 9H), 1.23-0.89 (m, 10H)

- 5 The tripeptide 11e (2.75 g, 3.99 mmol) was treated with 4N HCl/dioxane (20 mL) as described for compound 11c. The crude hydrochloride salt was dissolved in anhydrous DCM (20 mL). NMM (1.75 mL, 15.94 mmol) and acetic anhydride (752 µL, 7.97 mmol) were added successively. The reaction mixture was stirred 10 overnight at RT, then diluted with EtOAc. The organic layer was washed successively with 10% aqueous citric acid (2x), saturated aq. NaHCO3 (2x), water (2x) and brine (1x), dried (MgSO₄), filtered, and 15 evaporated to dryness to provide the crude tripeptide 11f as a white foam (2.48 g, 98% yield). MS (FAB) 632.4 MH^{+1} . ¹H NMR (CDCl₃), mainly one rotamer, δ 8.06(b d, J= 8Hz, 1H), 7.87 (b d, J= 8Hz, 1H), 7.83 (d, J=8Hz, 1H), 7.58-7.40 (m, 4H), 6.3620 (d, J= 9Hz, 1H), 6.01 (d, J= 9Hz, 1H), 5.94-5.83 (m,1H), 5.34-5.28 (m, 1H), 5.25-5.21 (m, 1H), 5.05 (d, J = 12Hz, 1H), 4.94 (d, J = 12Hz, 1H), 4.64-4.57 (m, 4H), 4.30-4.23 (m, 2H), 4.12-4.08 (m, 1H), 3.73 (dd, J = 4, 11Hz, 1H), 2.49-2.42 (m, 1H), 2.08-2.01 (m, 25 1H), 1.99 (s, 3H), 1.85-1.53 (m, 11H), 1.25-0.88 (m,
- The crude tripeptide **11f** (2.48 g, 3.93 mmol)was dissolved in an anhydrous mixture of CH₃CN: DCM (20 mL). Triphenylphosphine (53.5 mg, 0.200 mmol) and tetrakis(triphenylphosphine)-palladium (0) catalyst (117.9 mg, 0.102 mmol) were added successively, followed by pyrrolidine (353.9 µL, 4.24 mmol). The reaction mixture was stirred at room temperature for

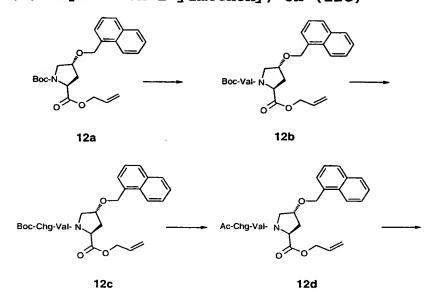
11H).

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Thereafter, the solvent was evaporated. The residue was dissolved in EtOAc and 10% aqueous citric acid , then, further washes twice more with 10% aqueous citric acid, water (2x), and brine (1x). The 5 organic layer was dried (MgSO₄), filtered and evaporated. The crude product was triturated in Et_2O : DCM (85:15) to provide after filtration the tripeptide 11g as a white solid (2.09 g, 90% yield). MS (FAB) 592.4 MH⁺ $614.3 \text{ (M+Na)}^{+}. \text{ }^{1}\text{H NMR (CDCl}_{3}),$ 10 mainly one rotamer, δ 8.08 (d, J= 8Hz, 1H), 7.93 (b d, J = 9Hz, 1H), 7.88 (b d, J = 8Hz, 1H), 7.82 (d, J =8Hz, 1H), 7.57-7.41 (m, 4H), 6.47 (d, J=8.5Hz, 1H), 5.05 (d, J = 12.5Hz, 1H), 4.94 (d, J = 12.5Hz, 1H), 4.73 (t, J= 9.5, 19Hz, 1H), 4.44-4.35 (m, 2H), 4.2615 (b s, 1H), 4.19 (d, J = 11.5Hz, 1H), 3.75 (dd, J = 4, 11Hz, 1H), 2.47 (b dd, J= 7.5, 13.5Hz, 1H), 2.20-2.11 (m, 1H), 2.04 (s, 3H), 1.88-1.41 (m, 11H), 1.30-0.80 (11H).

20 **Example 12**

Synthesis of "tripeptide segment" -Ac-Chg-Val-Pro(4(R)-naphthalen-1-ylmethoxy)-OH (12e)



√SDOCID: <WO___9907733A2_I_>

12e

Compound 12a (2.89 g, 7.02mmol) was treated with 4N HCl/dioxane (30 mL) as described for compound 11c. The crude hydrochloride salt was coupled to Boc-Val-OH (1.53 g, 7.73 mmol) with NMM (3.1 mL, 28.09 mmol) and TBTU (2.71 g, 8.43 mmol) in DCM (35 mL) for 3.5 h as described for compound 3 to provide the crude dipeptide 12b as an ivory oil-foam (ca. 3.60 g, 100% yield). MS (FAB) 509.3 MH⁻ 511.3 MH⁺ 533.2

10 (M+Na)⁺. ¹H NMR (CDCl₃) & 8.04 (b d, J= 8Hz, 1H), 7.87 (b d, J= 7Hz, 1H), 7.82 (d, J= 8Hz, 1H), 7.56-

7.87 (b d, J= 7Hz, 1H), 7.82 (d, J= 8Hz, 1H), 7.56-7.40 (m, 4H), 5.93-5.85 (m, 1H), 5.34-5.28 (m, 1H), 5.24-5.19 (m, 2H), 5.04 (d, J= 12Hz, 1H), 4.92 (d, J= 12Hz, 1H), 4.67-4.60 (m, 3H), 4.31-4.26 (m, 2H),

- 15 4.11-4.09 (m, 1H), 3.72 (dd, J= 4, 11Hz, 1H), 2.48-2.41 (m, 1H), 2.07-1.99 (m, 1H), 1.44-1.36 (m, 1H), 1.37 (s, 9H), 1.01 (d, J= 7Hz, 3H), 0.93 (d, J= 7Hz, 3H)
- The crude dipeptide **12b** (ca. 7.02 mmol) was treated with 4N HCl/dioxane (30 mL) as described for compound **11c**. The crude hydrochloride salt was coupled to Boc-Chg-OH H₂O (2.13g , 7.73mmol) with NMM (3.1 mL, 28.09 mmol) and TBTU (2.71 g, 8.43 mmol) in CH₂Cl₂ (35 mL) as described for compound 3 to provide the crude tripeptide **12c** as an ivory foam (ca. 4.6 g,

100% yield). MS (FAB) 648.5 MH 672.4 (M+Na) $^{+}$.

¹H NMR (CDCl₃) δ 8.06 (b d, J=8Hz, 1H), 7.87 (b d, J=

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7.5 Hz, 1H), 7.82 (b d , J= 8Hz, 1H), 7.57-7.40 (m, 4H), 6.46 (b d, J= 8.5Hz, 1H), 5.94-5.84 (m, 1H), 5.31 (dd, J= 1, 17Hz, 1H), 5.23 (dd, J= 1, 10.5Hz, 1H), 5.03 (d, J= 12Hz, 1H), 5.00-4.97 (m, 1H), 4.93 (d, J=, 12Hz, 1H), 4.63-4.59 (m, 4H), 4.29-4.27 (m, 1H), 4.10-4.07 (m, 1H), 3.92-3.86 (m, 1H), 3.72 (dd, J= 5, 11Hz, 1H), 2.48-2.41 (m, 1H), 2.10-1.99 (m, 1H), 1.76-1.57 (m, 6H), 1.43 (s, 9H), 1.20-0.92 (m, 6H), 1.00 (d, J= 7Hz, 3H), 0.93 (d, J= 7Hz, 3H).

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The crude tripeptide 12c (ca. 7.02mmol) was treated with 4N HCl/dioxane (30 mL) as described for compound 11c. The crude hydrochloride salt was further treated with acetic anhydride (1.33 mL, 14.05 mmol) and NMM (3.1 mL, 28.09 mmol) in CH₂Cl₂ (35 mL) as described for compound 11f. The crude product was flash purified (eluent:hexane:EtOAc;30:70) to provide the acetylated protected tripeptide 12d as a white foam (3.39 g, 81% yield over 3 steps). MS (FAB) 590.3

20 MH⁻ 592.4 MH⁺ 614.4 (M+Na)⁺

¹H NMR (CDCl₃), mainly one rotamer, δ 8.06 (d, J= 8Hz, 1H), 7.88 (b d, J= 8Hz, 1H), 7.83 (d, J= 8Hz, 1H), 7.58-7.41 (m, 4H), 6.37 (d, J= 9Hz, 1H), 5.97 (d, J= 8.5 Hz, 1H), 5.94-5.84 (m, 1H), 5.31 (dd, J= 1, 17Hz,

25 1H), 5.24 (dd, J= 1, 10.5 Hz, 1H), 5.05 (d, J= 12Hz, 1H), 4.94 (d, J= 12Hz, 1H), 4.66-4.57 (m, 4H), 4.31-4.22 (m, 2H), 4.11-4.05 (m, 1H), 3.73 (dd, J= 4.5, 11Hz, 1H), 2.50-2.43 (m, 1H), 2.09-2.01 (m, 2H), 2.00 (s, 3H), 1.68-1.55 (m, 5H), 1.15-0.89 (m, 6H), 0.99

30 (d, J=7Hz, 3H), 0.91 (d, J=7Hz, 3H).

The acetylated tripeptide **12d** (3.39 g, 5.73 mmol) was deprotected by tetrakis(triphenylphosphine) - palladium (0) catalyst (172.1 mg, 0.149 mmol) with

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triphenylphosphine (78.1 mg, 0.298 mmol) and pyrrolidine (516 μ L, 6.19 mmol) in a 1:1 mixture of anhydrous CH3CN: DCM (30 mL) as described for compound 11g. The crude light yellow foam product was 5 triturated in Et₂O : DCM (85:15)to provide after filtration the tripeptide 12e as an off-white solid (3.0 g; 95% yield). MS (FAB) 550.3 1 H NMR (CDCl₃) δ 8.08 (d, J= 8Hz, 1H), 8.04 (b d, J= 9Hz, 1H), 7.88 (b d, J=7.5Hz, 1H), 7.82 (d, J=8Hz, 1H), 7.58-7.37 (m, 5H), 5.05 (d, J= 12Hz, 1H), 4.94 10 (d, J= 12Hz, 1H), 4.61 (t, J= 9.5, 19.5Hz, 1H), 4.46-4.37 (m, 2H), 4.27 (b s, 1H), 4.17 (d, J=11Hz, 1H), 3.74 (dd, J= 4, 11Hz, 1H), 2.49 (b dd, J= 7.5, 13Hz,1H), 2.17-2.09 (m, 1H), 2.04 (s, 3H), 2.03-1.94 (m, 1H), 1.79 (b d, J=12.5Hz, 1H), 1.62-1.43 (m, 5H), 15 1.08-0.85 (m, 5H), 1.00 (d, J=7Hz, 3H), 0.90 (d, J=7Hz, 3H).

Example 13

20 General procedure for coupling reactions done on solid support.

The synthesis was done on a parallel synthesizer model ACT396 from Advanced ChemTech® with the 96 well block. Typically, 24 peptides were synthesized in parallel using standard solid-phase techniques. The starting Fmoc-Nva-Wang resin and the 1-(Fmoc-amino)cyclopropane carboxylic acid-Wang resin were prepared by the DCC/DMAP coupling method (Atherton, E; Scheppard, R.C. Solid Phase Peptide Synthesis, a Practical Approach; IRL Press: Oxford (1989); pp 131-148). Other amino acid-Wang resins were obtained from commercial sources.

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Each well was loaded with 100 mg of the starting resin (approximately 0.05 mmol). The resins were washed successively with 1.5 mL portions of NMP (1 X) and DMF (3 X). The Fmoc protecting group was removed 5 by treatment with 1.5 mL of a 25% v/v solution of piperidine in DMF for 20 min. The resins were washed with 1.5 mL portions of DMF (4 X), MeOH (3 X) and DMF The coupling was done in DMF (350 μ L), using 400 μ L (0.2 mmol) of a 0.5M solution of Fmoc-amino 10 acid/HOBt hydrate in DMF, 400 μ L (0.4 mmol) of a 0.5M solution of DIPEA in DMF and 400 μL (0.2 mmol) of a 0.5M solution of TBTU in DMF. After shaking for 1 h, the wells were drained, the resins were washed with 1.5 mL of DMF and the coupling was repeated once more 15 under the same conditions. The resins were then washed as described above and the cycle was repeated with the next amino acid.

The capping groups were introduced in two ways:

- In the form of a carboxylic acid using the protocol described above (for example acetic acid) or,
- As an acylating agent such as an anhydride or an acid chloride. The following example illustrates the capping with succinic anhydride: After the Fmoc deprotection and subsequent washing protocol, DMF was added (350 μL), followed by 400 μL each of a DMF solution of succinic anhydride (0.5 M, 0.2 mmol) and DIPEA (1.0 M, 0.4 mmol). The resins were stirred for 2 h and a recoupling step was performed.

At the end of the synthesis the resin was washed with 1.5 mL portions of DCM (3 x), MeOH (3 x), DCM (3 x), and were dried under vacuum for 2 h.

The cleavage from the resin and concomitant side chain deprotection was effected by the addition of 1.5 mL of a mixture of TFA, H₂O, DTT and TIS (92.5:

- 2.5: 2.5: 2.5). After shaking for 2.5 h, the resin was filtered and washed with 1.5 mL of DCM. The filtrates were combined and concentrated by vacuum centrifugation.
- 10 Each compound was purified by preparative reversed phase HPLC using a C18 column (22 mm by 500 mm). The product-containing fractions were identified by MALDI-TOF mass spectrometry, combined and lyophilized.

15

Example 14

Synthesis of compound 210 (Table 2)

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Using the experimental protocol described in Example 11 and starting with Fmoc-Cys(Trityl)-Wang resin, the above compound was obtained as a white solid (15.7 mg). MS (FAB) 849.2 (MH⁺), ¹H NMR (DMSO-d₆) δ 12.8 (broad s, 1H), 12.1 (broad s, 2H), 8.27 (d, J = 8 Hz, 1H), 8.17 (d, J = 7.5 Hz, 1H), 8.07 (d, J = 8 Hz, 1H), 8.00 (d, J = 8.4 Hz, 1H), 7.75 (d, J = 8.9 Hz, 1H), 7.34-7.27 (m, 5H), 4.54-4.39 (m, 5H), 4.31-4.18 (m, 4H), 4.10 (d, J = 11 Hz, 1H), 3.68 (dd, J = 3.9

Hz, J' = 10.8 Hz, IH), 2.90-2.82 (m, 1H), 2.78-2.70 (m, 1H), 2.67-2.42 (m, 4H), 2.21-2.17 (m, 3H), 2.00-1.85 (m, 3H), 1.83 (s, 3H), 1.80-1.67 (m, 1H), 1.67-1.42 (m, 6H), 1.15-0.95 (m, 4H), 0.88 (dd, J = 6.9 (m, 2H))

5 Hz, J' = 8.9 Hz, 6H).

Example 15

Synthesis of compound 215 (Table 2)

:

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215

The synthesis was carried out as shown below:

NSDOCID: <WO___9907733A2_I_>

15h

a) Synthesis of compound 15b:

1-(N-t-Boc-amino)cyclopropanecarboxylic acid (15a) (997 mg, 4.96 mmol) was dissolved in a mixture of anhydrous CH_2Cl_2 (25 mL) and THF (10 mL) . The 5 solution was cooled to 0°C , 2-trimethylsilylethanol (0.852 mL , 5.95 mmol), DMAP (121.1 mg, 0.991 mmol) and a DCC/CH₂Cl₂ solution (3.65 M; 1.63 mL, 5.95 mmol) were added successively. The reaction mixture 10 was stirred at 0°C for ca.4 h then at RT overnight. The white suspension was filtered through a diatomaceous earth pad. The pad was and rinsed with CH₂Cl₂. Filtrate and washing were evaporated to dryness. The residue was diluted with EtOAc and 15 sequentially washed with 10% aqueous citric acid (2x), saturated NaHCO₃ (2x), water (2x) and brine (1x). The organic layer was dried (MgSO₄), filtered, and evaporated to provide ester 15b as an oil (ca.1.5 g, 100%). ¹H NMR (CDCl₃) δ 5.08 (s, 1H), 4.20-4.16 20 (m, 2H), 1.57-1.43 (m, 2H), 1.45 (s, 9H), 1.17-1.12 (m, 2H), 1.00-0.94 (m, 2H), 0.04 (s, 9H).

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b) Synthesis of compound 15c:

Ester 15b (ca.700 mg, 2.33 mmol) was treated for 40 min at RT with 4N HCl/dioxane (11 mL). The solution was concentrated to dryness to provide the amine 5 hydrochloride as a white solid which was then subjected to the reaction conditions described in Example 6. The crude hydrochloride salt (950 mg, 2.55 mmol) and Boc-4(R)-(naphthalen-1ylmethoxy)proline (3) were dissolved in anhydrous 10 NMM (1.02 mL, 9.30 mmol) and HATU (1.06 g, CH_2Cl_2 . 2.79 mmol) were added successively and the mixture was stirred at RT. After 1.75 h, the reaction mixture was diluted with EtOAc and washed sequentially with 10% aq. citric acid (2x), saturated 15 aq. $NaHCO_3$ (2x), water (2x), and brine(1x). The EtOAc layer was dried (MgSO₄), filtered and concentrated to dryness to provide the crude dipeptide 15c as an offwhite foam (1.22 g). MS (FAB) 555.4 (MH^{+}) . ¹H NMR (CDCl₃); mixture of rotamers, δ 8.06-8.04 (m, 20 1H), 7.87-7.80 (m, 2H), 7.55-7.41 (m, 5H), 4.99-4.93 (m, 2H), 4.45-4.21 (m, 2H), 4.16-4.11 (m, 2H), 3.97-3.45 (m, 2H), 2.70-1.80 (m, 2H), 1.73-1.40 (m, 2H), 1.53 (s, (6/9) 9H), 1.44 (s, (3/9) 9H), 1.20-1.05 (m , 2H), 0.97-0.93 (m, 2H), 0.02 (s, 9H).

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c) Synthesis of compound 15d:

The crude dipeptide **15d** (ca. 2.20 mmol) was treated with 4N HCl/dioxane (11 mL) 40 min, RT and the resulting hydrochloride salt was coupled to Boc-Val-OH (525 mg, 2.42 mmol) with NMM (968 mL, 8.80 mmol) and HATU (1.00 g, 2.64 mmol) as described for compound **15c** (with the modification of 2.5 h coupling time). The crude tripeptide **15d** was obtained as an off-white foam (1.5 g). MS (FAB) 654.4 (MH⁺). ¹H

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NMR (CDCl₃) δ 8.05-8.02 (m, 1H), 7.87-7.80 (m, 2H), 7.55-7.40 (m, 5H), 7.30-7.28 (m, 1H), 5.19-4.62 (m, 4H), 4.41-3.70 (m, 1H), 4.35-4.27 (m, 1H), 4.09-3.95 (m, 1H) 3.73-3.62 (m, 2H), 2.69-2.60 (m, 1H), 2.14-1.94 (m, 2H), 1.55-1.38 (m, 2H), 1.39 (s, 9H), 1.22-1.18 (m, 1H), 1.11-1.07 (m, 1H), 0.98-0.90 (m, 8H), 0.02 (s, 9H).

d) Synthesis of compound 15e:

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- The crude tripeptide **15d** (ca. 2.20 mmol) was treated with 4N HCl/dioxane (11 mL) 40 min, RT and the resulting hydrochloride salt was coupled to Boc-Chg-OH (622 mg, 2.42 mmol) with NMM (968 mL, 8.80 mmol) and TBTU (847 mg, 2.64 mmol) as described for
- compound **15c** (with the modifications of using TBTU as a coupling agent and stirring at RT for ca. 64 h prior to work-up). The foam-like residue was purified by flash chromatography (eluent: hexane: EtOAc; 6:4) to provide the tetrapeptide **15e** as a white foam
- 20 (710.8 mg; 41% yield over 3 steps). MS (FAB) 793.4 (MH⁺). 1 H NMR (CDCl₃) δ 8.07-8.05 (m, 1H), 7.87-7.80 (m, 2H), 7.57-7.41 (m, 4H), 7.35 (s, 1H), 6.72-6.64 (m, 1H), 5.02-4.95 (m, 3H), 4.68-4.62 (m, 2H), 4.43-4.40 (m, 1H), 4.15-4.00 (m, 2H), 3.96-3.93 (m,
- 25 2H), 3.68 (dd, J= 11, J'= 5 Hz, 1H), 2.62-2.56 (m, 1H), 2.16-2.00 (m, 2H), 1.70-1.54 (m, 6H), 1.49-1.42 (m, 2H), 1.43 (s, 9H), 1.14-1.02 (m, 5H), 0.95-0.88 (m, 10H), 0.02 (s, 9 H).

30 e) Synthesis of compound 15f:

Tetrapeptide **15e** (168.1 mg, 0.212 mmol) was treated with 4N HCl/dioxane solution (2 mL) and the resulting hydrochloride salt was coupled to Boc-(D)Glu(OTMSE)-OH (81.0 mg, 0.233 mmol) with NMM (94 mL, 0.848 mmol)

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and TBTU (81.7 mg, 0.254 mmol) as described for compound **15e** (with the modification of 17 h coupling time). The crude pentapeptide **15f** was obtained as an off-white foam (220 mg, 0.212 mmol) . MS (FAB)

- 5 1022.8 (MH⁺) 1044.8 (MNa⁺). ¹H NMR (CDCl₃) δ 8.07-8.05 (m, 1H), 7.88-7.81 (m, 2H), 7.57-7.41 (m, 4H), 7.29 (s, 1H), 6.70-6.55 (m, 2H), 5.45-5.35 (m, 1H), 4.99-4.98 (m, 2H), 4.66-4.57 (m, 2H), 4.44-4.40 (m, 1H), 4.30-4.01 (m, 5H), 3.91 (dd, J= 11, J'= 4 Hz, 1H),
- 10 3.76-3.62 (m, 2H), 2.62-2.56 (m, 1H), 2.50-2.30 (m, 3H), 2.18-2.09 (m, 2H), 2.06-1.90 (m, 2H), 1.67-1.53 (m, 4H), 1.50-1.42 (m, 4H), 1.43 (s, 9H), 1.14-0.86 (m, 10H), 0.93 (d, J= 7 Hz, 3H), 0.87 (d, J= 7 Hz, 3H), 0.04 (s, 9H), 0.02 (s, 9H).

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f) Synthesis of compound 15g:

The crude pentapeptide 15f (ca. 0.212 mmol) was treated with 4N HCl/dioxane solution (2.5 mL) 40 min, RT and the resulting hydrochloride salt was coupled to Boc-Asp(OTMSE)-OH (77.8 mg, 0.233 mmol) with NMM (93 mL, 0.848 mmol) and TBTU (81.7 mg, 0.254 mmol) as described for compound 15e (with the modification of 2.5 h coupling time). The crude hexapeptide 15g was obtained as an ivory foam (278 mg, 0.212 mmol). MS (FAB) 1237.5 (MH⁺) 1259(MNa⁺).

g) Synthesis of compound 15h:

The crude hexapeptide **15g** (ca. 0.2 mmol) was treated for 40 min at RT with 2.5 mL 4N HCl/dioxane solution.

Concentration to dryness provided the amine hydrochloride as a white solid. The crude hydrochloride salt was dissolved in anhydrous DMF (2.5 mL) and treated successively with pyridine (377 µL, 4.66 mmol) and acetic anhydride (378 µL, 4.01

mmol). The reaction mixture was stirred overnight at RT then poured into brine and extracted with EtOAc (3x). The combined organic layer was washed successively with 10% aqueous citric acid (2x), 5 saturated NaHCO₃ (2x), water (2x), and brine (1x). The organic layer was dried (MgSO₄), filtered and evaporated to dryness. The foamy residue was purified by flash chromatography (eluent : hexane : EtOAc; 3:7) to provide the acetylated hexapept 15h as an off-white foam (78.5 mg, 31% yield over 3 steps). 10 1179.6 (MH^{+}) 1201.5 (MNa^{+}) . ¹H NMR $(CDCl_{3})$ δ (FAB) 8.11-8.09 (m, 1H), 7.86-7.79 (m, 2H), 7.55-7.41 (m, 5H), 7.28 (s, 1H), 7.02-6.96 (m, 2H), 6.70-6.68 (m, 1H), 5.13-5.10 (m, 1H), 4.96-4.91 (m, 2H), 4.58-4.41 15 (m, 4H), 4.22-4.08 (m, 8H), 3.77 (dd, J= 10.5, J'= 5)Hz, 1H), 3.09 (dd, J=18, J'=4 Hz, 1H), 2.76 (dd, J=17.5, J' = 8 Hz, 1H), 2.51-2.20 (m, 3H), 2.12-2.08 (m, 2H), 2.09 (s, 3H), 1.73-1.53 (m, 8H), 1.27-1.09 (m, 7H), 1.01-0.85 (m, 8H), 0.98 (d, J=6.5 Hz, 3H), 20 0.97(d, J= 6 Hz, 3H), 0.04 (s, 9H), 0.03 (s, 9H),

h) Synthesis of compound 215:

0.01 (s, 9H).

The acetylated hexapeptide 15h (76.5 mg, 0.065 mmol)

was dissolved in anhydrous THF (2 mL), a TBAF

solution (1M in THF; 389 µL, 0.389 mmol) was added

and the mixture was stirred at RT for 16 h. The

solution was concentrated under vacuum and the

residue was dissolved in glacial acetic acid,

filtered through a Millipore* Millex*-HV 0.45 µm

filter unit and injected onto an equilibrated Whatman

Partisil* 10-ODS-3 (2.2 x 50cm) C18 reverse phase

column. Purification program: Linear Gradient at 15

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mL/min, λ 230 nm, program at 5% A for 10 min, 5-30% A in 10 min, at 30% A for 10 min, 30-60% A in 90 min A:0.06% TFA/CH₃CN; B:0.06% TFA/H₂O. Fractions were analyzed by analytical HPLC. The product collected was lyophilized to provide the hexapeptide acid 215 as a white amorphous solid (26.9 mg; contains 41% by weight of tetrabutylammonium salts, 28% yield). $879.4 \, (MH^{+}) \, 901.3 \, (MNa^{+})$. In order to remove the tetrabutylammonium salt, the above product (ca.18 mg) was dissolved in EtOAc and washed with 10% HCl (2x). The EtOAc layer was evaporated, then lyophilized with water to provide the salt -free product as a white amorphous solid (3.8 mg , 36% yield). 1 H NMR (DMSO-d₆) δ 8.39 (s, 1H), 8.10-7.81 (m, 7H), 7.57-7.45 (m, 4H), 5.07-4.87 (m, 2H), 4.55-4.00 (m, 7H), 3.76-3.71 (m, 1H), 2.67-2.62 (m, 1H), 2.33-2.10 (m, 3H), 2.05-1.42 (m, 8H), 1.79 (s, 3H), 1.38-0.71 (m, 1H), 0.89 (d, J=6.68 Hz, 3H), 0.86 (d, J=6.36 Hz, 3H).

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Example 16

Synthesis of compound 214 (Table 2):

214

For the synthesis of compound **214** the procedure described in example 15 was followed, using Boc-4(R)-(naphthalen-2-ylmethoxy)proline for the introduction

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PCT/CA98/00765

of the P2 fragment and with different protecting groups at the side chain carboxylic acid residues.

The synthesis is described below:

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\SDOCID: <WO___9907733A2_I_>

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a) Synthesis of compound 16b:

At 0°C, benzyl bromide (5.74 mL, 48.3 mmol) was added to a mixture of Boc-norvaline (16a) (10.0 g, 46.0 mmol) and DBU (7.57 mL, 50.6 mmol) in acetonitrile 5 (200 mL). After stirring at RT for 20 h, the solution was concentrated and the residue dissolved in ether. The organic solution was washed sequentially with 10% aqueous citric acid (2x), saturated aqueous. NaHCO₃ (2x) and brine (1x), dried $(MgSO_4)$, filtered and concentrated to give the desired benzyl ester 16b as 10 a colorless oil (13.7 g, 97% yield). $^{1}\text{H NMR}$ (CDCl3) δ 7.40-7.32 (m, 5H), 5.16 (dd, J = 26.7, J' = 12.4 Hz, 2H), 4.99 (d, J = 7.9 Hz, 1H), 4.35-4.32 (m, 1H), 1.82-1.73 (m, 1H), 1.66-1.57 (m, 1H), 1.43 (s, 9H), 15 1.41-1.32 (m, 2H), 0.90 (t, J = 7.3 Hz, 3H).

b, c, d, e, f, g) Synthesis of compound 16h:

The above Boc-Nva benzyl ester (121 mg, 0.48 mmol) was subjected to the same sequence of reactions as described in example 7. However, for the introduction of P2 (step b) Boc-4(R)-(naphthalen-2-ylmethoxy)proline was used. Also, for the introduction of P5 (step e) and P6 (step f) the corresponding Boc-D-Glu-OH and Boc-Asp-OH residues were protected as benzyl esters at the carboxylic acid side chain.

h) Synthesis of compound 214:

To a solution of hexapeptide **16h** (ca. 0.210 mmol) in ethanol (3 mL) was added 10% palladium on charcoal (10 mg) and ammonium acetate (10 mg). The mixture was stirred under an atmosphere of hydrogen for 5 h, then filtered through a Millipore®: Millex®-HV 0.45 µm filter unit and injected onto an equilibrated Whatman

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Partisil® 10-ODS-3 (2.2 x 50 cm) C18 reverse phase column. Purification program: Linear Gradient at 15 mL/min, λ 230 nm, at 5% to 50% A in 60 min A: 0.06% TFA/CH₃CN; B: 0.06% TFA/H₂O. Fractions were analyzed 5 by HPLC . The collected product was lyophilized to provide 214 as a white solid (20 mg, 0.02 mmol). MS (FAB) 895.5 (MH⁺). ¹H NMR (CDCl₃) δ 8.16 (d, J = 7.6 Hz, 1H), 8.11 (d, J = 8 Hz, 1H), 8.09 (d, J = 8)Hz, 1H), 7.98 (d, J = 9 Hz, 1H), 7.91-7.88 (m, 3H), 10 7.85 (s, 1H), 7.77 (d, J = 9 Hz, 1H), 7.51-7.46 (m,3H), 4.70 (d, J = 12 Hz, 1H), 4.60 (d, J = 12 Hz, 1H), 4.53-4.45 (m, 2H), 4.33-4.10 (m, 6H), 3.69 (dd, J = 19, J' = 4.4 Hz, 1H), 2.66-2.60 (m, 1H), 2.49-2.43(m, 1H), 2.21-2.18 (m, 3H), 2.07-1.94 (m, 3H), 1.82 15 (s, 3H), 1.76-1.33 (m, 10H), 1.04-0.86 (m, 15H).

Example 17

Synthesis of compound 221 (Table 2):

20 . 221

Mono-benzylsuccinic acid (prepared as described in: Bischoff, V. et al., Chem.Ber. (1902), 35, 4078) (27 mg, 0.134 mmol) was stirred in acetonitrile (2 mL) with TBTU (52 mg, 0.160 mmol) and NMM (47 mg, 0.469 mmol) for 5 min. To this mixture, the hydrochloride salt of the appropriate tetrapeptide (prepared as described for compound **16e** but using isoleucine instead of cyclohexylglycine and 4(R)-(naphthalen-1-

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ylmethoxy)proline instead of a 4(R)-(naphthalen-2-ylmethoxy)proline (97.0 mg, 0.134 mmol) was added. The mixture was stirred at RT for 2.5 h. Ethyl acetate was added and the mixture was washed with 10% aqueous citric acid (2x), with saturated aqueous NaHCO₃ (2x) and brine (1x), dried (MgSO₄), filtered and concentrated to afford the protected tetrapeptide as a yellow oil.

- The above compound (ca. 0.134 mmol) was dissolved in ethanol (3 mL) and ammonium acetate (10 mg) and 20% palladium hydroxide on activated carbon (30 mg) were added. The mixture was stirred under 1 atmosphere of hydrogen for 18 h, then filtered through a
- Millipore[®]: Millex[®]-HV 0.45 μm filter unit and injected onto an equilibrated Whatman Partisil 10-ODS-3 (2.2 x 50 cm) C18 reverse phase column. Purification program: Linear Gradient at 15 mL/min, λ 230 nm, 5% A for 10 min, 5-60% A in 60 min (A:
- 0.06% TFA/CH₃CN; B: 0.06% TFA/H₂O). Fractions were analyzed by HPLC . The collected product was lyophilized to provide **221** as a white solid (21 mg). MS (FAB) 683 (MH⁺). 1 H NMR (DMSO-d₆) δ 8.12 (d, J = 7.6 Hz, 1H), 8.07-8.03 (m, 1H), 7.96-7.81 (m, 4H),
- 25 7.59-7.51 (m, 3H), 7.55 (t, J = 8.0 Hz, 1H), 4.90 (d, J = 8 Hz, 1H), 4.82 (d, J = 8 Hz, 1H), 4.45 (t, J = 8.0 Hz, 1H), 4.36-4.31 (m, 2H), 4.24-4.12 (m, 3H), 3.74-3.68 (m, 1H), 2.43-2.31 (m, 4H), 2.24-2.18 (m, 1H), 2.01-1.92 (m, 2H), 1.67-1.51 (m, 3H), 1.42-1.32
- 30 (m, 3H), 1.14-0.96 (m, 1H), 0.93-0.67 (m, 15H).

Example 18

The following description is an example of a compounds of formula I wherein Q is $CH_2C(O)$.

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Preparation of compound 413 (Table 4)

NSDOCID: <WO___9907733A2_I_>

Compound 18b

- 1) To cyclohexylacetic acid (18a) (8g, 56.25 mmol) in DCM (160 mL) at room temperature was added the oxalyl chloride (6.4 mL, 73.14 mmol) and 2 drops of DMF. The reaction mixture was stirred at room temperature for 1h, then concentrated under reduced pressure to give cyclohexylacetyl chloride.
- 2) The chiral auxiliary, (4S)-(-)-4-isopropyl-2-oxazolidinone, (7.63g, 59.06 mmol) was dissolved in THF (200 mL) and cooled to -78°C. N-butyllithium (1.6M) in hexane (36.9 mL, 59.06 mmol) was added slowly (over a 10 min period). The mixture was stirred at -78°C for 30 min (formed a gel). The aformentioned cyclohexylacetyl chloride was added in THF (50 mL) at -78°C. The reaction mixture was stirred at -78°C for 30 min and then at 0°C for 1h. The reaction was quenched by adding an aqueous

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solution of NH₄Cl (16 mL). The reaction mixture was concentrated under reduced pressure. Et₂O (300 mL) was added. The organic phase was separated and washed with a 10% aqueous solution of citric acid (2 x 200 mL), a saturated aqueous solution of NaHCO3 (2 x 200 mL) and brine (200 mL), dried, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, 40-60μ, 60 x 100 mm, $9/1 \rightarrow 8/\rightarrow 2$, hexane/EtOAc to give 10 compound 18b as a colorless oil (11.3 g, 79% yield). ¹H NMR (CDCl₃) δ 4.40-4.36 (m, 1H), 4.20 (dd, J = 8.3Hz, J=9.1Hz, 1H), 4.13 (dd, J=2.9Hz, 9.1Hz, 1H), 2.86 (dd, J = 6.4 Hz, 15.7 Hz, 1 H), 2.65 (dd, J =7.1Hz, 15.7Hz, 1H), 2.35-2.27 (m, 1H), 1.83-1.76 (m, 15 1H), 1.70-1.57 (m, 5H), 1.26-0.90 (m, 5H), 0.85 (d, J = 7.0Hz, 3H), 0.81 (d, J = 6.7Hz, 3H).

Compound 18c

To a solution of compound 18b (11.3 g, 44.68 mmol) in 20 THF (125 mL) at -78°C was added a NaHMDS solution (1M in THF, 49.2 mL, 49.15 mmol). The reaction mixture was stirred at -78°C for 1.5 h. A solution of tertbutyl bromoacetate (8.67 mL, 53.62 mmol) in THF (25 mL) was added at -78°C. The mixture was stirred at 25 that temperature for 3h. A saturated aqueous solution of NH₄Cl solution (33 mL) was added slowly. The cold bath was removed and the mixture was stirred at room temperature for 10 min. The THF was removed. EtOAc was added (200 mL). The organic phase was 30 separated, washed serially with a saturated aqueous solution of $NaHCO_3$ (200 mL), H_2O (200 mL), aqueous 1NHCl solution (200 mL) and brine (200 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by trituration

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with Et_2O giving compound **18c** as a white solid (12.65g, 77% yield).

¹H NMR (DMSO-d₆) δ 4.61-4.53 (m, 3H), 4.27-4.25 (m, 1H), 2.84-2.66 (m, 2H), 2.55-2.41 (m, 1H), 1.89-1.76 (m, 6H), 1.58 (s, 9H), 1.35-1.31 (m, 4H), 1.14-1.04 (m, 7H).

Compound 18d

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To an ice-cold solution of compound **18c** (12.2 g, 33.28 mmol) in a mixture of THF/H₂O (3/1 mixture, 495 mL/165 mL) was added H₂O₂ (30%, 15.1 mL, 133.1 mmol), followed by a slow addition of LiOH-H₂O (2.79 g, 66.56 mmol). The reaction mixture was stirred at 0°C for 1 h, then at RT overnight. The mixture was

- cooled to 0°C and a 1.5N aqueous solution of Na₂SO₃ was added slowly to decompose excess peroxide (monitored by KI paper). The mixture was concentrated under reduced pressure, the residual aqueous solution was washed with DCM (2 x 150 mL).
- The aqueous layer was made acidic with a 10% aqueous solution of citric acid. The mixture was extracted with EtOAc (3 x 200 mL). The combined organic phase were washed with brine (200 mL), dried (MgSO₄), filtered and concentrated under reduced pressure.
- Compound **18d** was obtained as a colorless oil (8.38g, 98% yield).

¹H NMR (CDCl₃) δ 2.71-2.66 (m, 1H), 2.59 (dd, J = 10.8Hz, 16.0Hz, 1H), 2.36 (dd, J = 3.8Hz, 16.0Hz, 1H), 1.78-1.57 (m, 6H), 1.41 (s, 9H), 1.30-0.98 (m, 5H).

Compound 18f

1) The corresponding Boc derivative of compound **18e** (1.63 g, 2.74 mmol) was treated with HCl 4N/dioxane

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(14 mL, 54.91 mmol) at RT for 1 h. The reaction mixture was concentrated under reduced pressure. 5% aqueous solution of Na_2CO_3 (25 mL) was added to the residue and the resulting solution was stirred vigorously for 5 min. EtOAc was added (75 mL). 5 two resulting phases were separated. The organic phase was washed with brine (50 mL), dried (MgSO₄), filtered and concentrated under reduced pressure to give 18e which was used as such for the next step. 10 2) To the amino tripeptide in DMF (5 mL) at RT was added compound 18d (739 mg, 288 mmol) in DMF (5 mL), followed by DIPEA (1.43 mL, 8.24 mmol) and TBTU (502 mg, 2.88 mmol). The reaction mixture was stirred at RT overnight. EtOAc was added (125 mL). The organic 15 phase was separated, washed with a saturated aqueous solution of $NaHCO_3$ (100 mL), H_2O (100 mL) and brine (100 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, $40-60\mu$, 40×125 mm, 20 $6/4 \rightarrow 5/5$ hexane/EtOAc) to give the tert-butyl ester compound 18f as a white foam (1.18g, 59% yield). ¹H NMR (CDCl₃) δ 8,06 (d, J = 8.3Hz, 1H), 7.86 (d, J = 7.6Hz, 1H), 7.81 (d, J = 8.3Hz, 1H), 7.55-7.40 (m, 4H), 7.35 (s, 1H), 6.28 (d, J = 8.9Hz, 1H), 5.86-5.7925 (m, 1H), 5.24 (dd, J = 1.6Hz, 17.2Hz, 1H), 5.17 (dd, J = 1.6Hz, 17.2Hz, 1H)J = 1.3Hz, J = 10.5Hz, 1H), 4.98 (ABq, $\Delta v = 18.7Hz$, J =12.1Hz, 2H), 4.67-4.51 (m, 4H), 4.41-4.38 (m, 1H), 3.99 (dd, J = 3.8 Hz, 10.8 Hz, 1H), 2.64-2.59 (m, 2H),2.42-2.38 (m, 2H), 2.10-1.95 (m, 2H), 1.68-1.53 (m, 30 9H), 1.43-1.41 (m, 1H), 1.42 (s, 9H), 1.15-1.04 (m, 4H), 0.97-0.91 (m, 8H).

Compound 18h

To the commercially available 3-[benzyl-2-methoxycarbonylethyl)amino]propionic acid methyl ester (18g) (2 g, 7.16 mmol) in MeOH (24 mL), was added the palladium catalyst (Pd/C 10%, 500 mg, 25 % w/w). The reaction mixture was stirred under a nitrogen atmosphere (balloon) for 18 h. The mixture was filtered through diatomaceous ester and the filter pad was washed with MeOH (20 mL). The MeOH (filtrate plus washing) was evaporated to give 1.2g (89% yield) of compound 18h as a pale yellow oil. This product was used as such for the next step.

Compound 18i

- 1) The t-butyl ester compound 18f, (1.18 g, 1.62 mmol) was treated with 4N HCl in dioxane (8.5 mL, 32.4 mol) at RT for 6 h. The mixture was concentrated under reduced pressure, and then co-evaporated with benzene/Et₂O to give 1.04 g of the corresponding acid as a beige foam (95% yield).
 - 2) To the latter acid (200 mg, 0.29 mmol) in DMF (1 mL) at RT was added the amine (compound 18h, 59 mg, 0.31 mmol) in DMF (2 mL), followed by DIPEA (154 μ L,
- 0.89 mmol) and TBTU (100 mg, 0.31 mmol). The reaction mixture was stirred at RT for 72 h. EtOAc (125 mL) was added. The organic phase was separated, washed with a saturated aqueous solution of NaHCO₃ (75 mL), H₂O (75 mL) and brine (75 mL), dried
- MgSO₄), filtered and concentrated under reduced pressure. The product was purified by flash chromatography (silica gel, 40-60μ, 20 x 100 mm, 8/2 EtOAc/hexane to give compound **18i** as a yellow oil (82 mg, 33% yield).

95 MS (ESI) 869.3 (M+Na)⁺, 845.4 (M-H)⁻.

Compound 413

An aqueous 1M solution of NaOH (774 μ L, 0.774 mmol) 5 was added to a solution of compound 18i (82 mg, 0.097 mmol) in a mixture of THF/MeOH (1/1, 1 mL each). reaction mixture was stirred at RT for 18 h. H₂O was added (15 mL). The aqueous phase was separated and washed with DCM (3 \times 15 mL). The aqueous phase was 10 made acidic (pH 3) by adding an aqueous solution of 1N HCl. The mixture was extracted with EtOAc (3 x 15 mL). The organic phase was washed with brine (25 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by 15 preparative HPLC (5% \rightarrow 53% MeCN in 60 min) to give compound 413 as a white lyophilized solid (31 mg, 41% yield).

MS (ESI) 779.3 (M+H)⁺, 801.3 (M+Na)⁺, 777.3 (M-H)⁻

1 H NMR (DMSO-d₆) δ 8.38 (S, 1H), 8.06 (d, J = 8.3Hz, 1H), 7.93 (d, J = 7.6Hz, 1H), 7.86 (d, J = 8.3Hz, 1H), 7.74 (d, J = 8.6Hz, 1H), 7.57-7.44 (m, 5H), 5.01 (d, J = 12.1Hz, 1H), 4.89 (d, J = 12.1Hz, 1H), 4.35-4.31 (m, 2H), 4.25 (dd, J = 7.9Hz, 8.3Hz, 1H), 4.18 (d, J = 11.1Hz, 1H), 3.80-3.49 (m, 3H), 3.37-3.34 (m, 2H), 2.63-2.61 (m, 2H), 2.56-2.52 (m, 1H), 2.39-2.35 (m, 2H), 2.25-2.20 (m, 2H), 2.05-1.91 (m, 2H), 1.62-1.59 (m, 1H), 1.41-1.22 (m, 5H), 0.96-0.73 (m, 16H).

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Example 19

RECOMBINANT HCV NS3 PROTEASE RADIOMETRIC ASSAY

a) Cloning, expression and purification of the recombinant HCV NS3 protease type 1b

Serum from an HCV-infected patient was obtained through an external collaboration (Bernard Willems MD, Hôpital St-Luc, Montréal, Canada and Dr. Donald 10 Murphy, Laboratoire de Santé Publique du Québec, Ste-Anne de Bellevue, Canada). An engineered full-length cDNA template of the HCV genome was constructed from DNA fragments obtained by reverse transcription-PCR (RT-PCR) of serum RNA and using specific primers selected on the basis of homology between other 15 genotype 1b strains. From the determination of the entire genomic sequence, a genotype 1b was assigned to the HCV isolate according to the classification of Simmonds et al. (J. Clin. Microbiol. (1993), 31, 20 1493-1503.). The amino acid sequence of the nonstructural region, NS2-NS4B, was shown to be greater than 93% identical to HCV genotype 1b (BK, JK and 483 isolates) and 88% identical to HCV genotype 1a (HCV-1 isolate). A DNA fragment encoding the polyprotein 25 precursor (NS3/NS4A/NS4B/NS5A/NS5B) was generated by PCR and introduced into eucaryotic expression vectors. After transient transfection, the polyprotein processing mediated by the HCV NS3 protease was demonstrated by the presence of the mature NS3 protein using Western blot analysis. 30 mature NS3 protein was not observed with expression of a polyprotein precursor containing the mutation S1165A, which inactivates the NS3 protease,

confirming the functionality of the HCV NS3 protease.

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The DNA fragment encoding the recombinant HCV NS3 protease (amino acid 1027 to 1206) was cloned in the pET11d bacterial expression vector. The NS3 protease expression in *E. coli* BL21(DE3)pLysS was induced by incubation with 1 mM IPTG for 3 h at 22°C. A typical fermentation (18 L) yielded approximately 100 g of wet cell paste. The cells were resuspended in lysis buffer (3.0 mL/g) consisting of 25 mM sodium

phosphate, pH 7.5, 10% glycerol (v/v), 1 mM EDTA, 0.01% NP-40 and stored at -80°C. Cells were thawed and homogenized following the addition of 5 mM DTT. Magnesium chloride and DNase were then added to the homogenate at final concentrations of 20 mM and

15 20 μ g/mL respectively. After a 25 min incubation at 4°C, the homogenate was sonicated and centrifuged at 15000 x g for 30 min at 4°C. The pH of the supernatant was then adjusted to 6.5 using a 1M sodium phosphate solution.

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An additional gel filtration chromatography step was added to the 2 step purification procedure described in WO 95/22985 (incorporated herein by reference). Briefly, the supernatant from the bacterial extract was loaded on a SP HiTrap® column (Pharmacia) 25 previously equilibrated at a flow rate of 2 mL/min in buffer A (50 mM sodium phosphate, pH 6.5, 10% glycerol, 1 mM EDTA, 5 mM DTT, 0.01% NP-40). column was then washed with buffer A containing 0.15 30 M NaCl and the protease eluted by applying 10 column volumes of a linear 0.15 to 0.3 M NaCl gradient. protease-containing fractions were pooled and diluted to a final NaCl concentration of 0.1 M. The enzyme was further purified on a HiTrap® Heparin column

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(Pharmacia) equilibrated in buffer B (25 mM sodium phosphate, pH 7.5, 10% glycerol, 5 mM DTT, 0.01% NP-40). The sample was loaded at a flow rate of 3 The column was then washed with buffer B containing 0.15 M NaCl at a flow rate of 1.5 mL/min. Two step washes were performed in the presence of buffer B containing 0.3 or 1M NaCl. The protease was recovered in the 0.3M NaCl wash, diluted 3-fold with buffer B, reapplied on the $HiTrap^{\otimes}$ Heparin column and eluted with buffer B containing 0.4 M NaCl. the NS3 protease-containing fractions were applied on a Superdex 75 HiLoad® 16/60 column (Pharmacia) equilibrated in buffer B containing 0.3 M NaCl. purity of the HCV NS3 protease obtained from the pooled fractions was judged to be greater than 95% by SDS-PAGE followed by densitometry analysis.

The enzyme was stored at $-80\,^{\circ}\text{C}$ and was thawed on ice and diluted just prior to use.

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b) RECOMBINANT HCV NS3 PROTEASE RADIOMETRIC ASSAY

The substrate used for the HCV NS3 protease radiometric assay, DDIVPC-SMSYTW, is cleaved between the cysteine and the serine residues by the enzyme. The sequence DDIVPC-SMSYTW corresponds to the NS5A/NS5B natural cleavage site in which the cysteine residue in P2 has been substituted for a proline. The peptide substrate DDIVPC-SMSYTW and the tracer biotin-DDIVPC-SMS[125I-Y]TW were incubated with the recombinant NS3 protease in the absence or in the presence of inhibitors. The separation of substrate from products was performed by adding avidin-coated agarose beads to the assay mixture followed by

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filtration. The amount of SMS[125I-Y]TW product found in the filtrate (with or without inhibitor) allowed for the calculation of the percentage of substrate conversion and of the percentage of inhibition.

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A. Reagents

Tris and Tris-HCl (UltraPure) were obtained from Life Technologies. Glycerol (UltraPure), MES and BSA were purchased from Sigma[®]. TCEP was obtained from Pierce, DMSO from Aldrich[®] and NaOH from Anachemia[®].

Assay buffer: 50 mM Tris-HCl, pH 7.5, 30% (w/v) glycerol, 2% (w/v) CHAPS, 1 mg/mL BSA, 1 mM TCEP (TCEP added just prior to use from a 1 M stock solution in water).

Substrate: DDIVPC-SMSYTW, 25 μ M final concentration (from a 2 mM stock solution in DMSO stored at -20°C to avoid oxidation).

Tracer: reduced mono-iodinated substrate(biotin-DDIVPC-SMS[125 I-Y]TW) (\approx 1 nM final concentration).

25 HCV NS3 protease type 1b, 25 nM final concentration (from a stock solution in 50 mM sodium phosphate, pH 7.5, 10% glycerol, 300 mM NaCl, 5 mM DTT, 0.01% NP-40).

30 B. Protocol

The assay was performed in a 96-well polypropylene plate. Each well contained:

20 μL substrate/tracer in assay buffer;

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- $\circ~10~\mu L$ ± inhibitor in 20% DMSO/assay buffer;
- \circ 10 μ L NS3 protease 1b.

Blank (no inhibitor and no enzyme) and control (no inhibitor) were also prepared on the same assay plate.

The enzymatic reaction was initiated by the addition of the enzyme solution and the assay mixture was incubated for 60 min at 23°C under gentle agitation. Twenty (20) µL of 0.025 N NaOH were added to quench the enzymatic reaction.

Twenty (20) µL of avidin-coated agarose beads

(purchased from Pierce®) were added in a Millipore®

MADP N65 filtration plate. The quenched assay mixture

was transferred to the filtration plate, and
incubated for 60 min at 23°C under gentle agitation.

The plates were filtered using a Millipore® MultiScreen Vacuum Manifold Filtration apparatus, and 40 μ L of the filtrate was transferred to an opaque 96-well plate containing 60 μ L of scintillation fluid per well.

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The filtrates were counted on a Packard® TopCount instrument using a ¹²⁵I-liquid protocol for 1 minute. The %inhibition was calculated with the following equation:

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100-[(counts_{inh}-counts_{blank})/(counts_{ct1}-counts_{blank}) x 100]

101

A non-linear curve fit with the Hill model was applied to the inhibition-concentration data, and the 50% effective concentration (IC_{50}) was calculated by the use of SAS software (Statistical Software System; SAS Institute, Inc., Cary, N.C.).

Example 20

RECOMBINANT HCV NS3 PROTEASE/NS4A COFACTOR PEPTIDE RADIOMETRIC ASSAY

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The enzyme was cloned, expressed and prepared according to the protocol described in Example 19. The enzyme was stored at -80°C, thawed on ice and diluted just prior to use in the assay buffer containing the NS4A cofactor peptide.

The substrate used for the NS3 protease/NS4A cofactor peptide radiometric assay, DDIVPC-SMSYTW, is cleaved between the cysteine and the serine residues by the 20 enzyme. The sequence DDIVPC-SMSYTW corresponds to the NS5A/NS5B natural cleavage site in which the cysteine residue in P2 has been substituted for a proline. The peptide substrate DDIVPC-SMSYTW and the tracer biotin-DDIVPC-SMS[125I-Y]TW are incubated with the 25 recombinant NS3 protease and the NS4A peptide cofactor KKGSVVIVGRIILSGRK (molar ratio enzyme: cofactor 1:100) in the absence or presence of inhibitors. The separation of substrate from products is performed by adding avidin-coated agarose beads to the assay mixture followed by filtration. The amount 30 of SMS[125I-Y]TW product found in the filtrate allows for the calculation of the percentage of substrate conversion and of the percentage of inhibition.

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A. Reagents

Tris and Tris-HCl (UltraPure) were obtained from Life Technologies. Glycerol (UltraPure), MES and BSA were purchased from Sigma[®]. TCEP was obtained from Pierce, DMSO from Aldrich[®] and NaOH from Anachemia[®].

Assay buffer: 50 mM Tris HCl, pH 7.5, 30% (w/v) glycerol, 1 mg/mL BSA, 1 mM TCEP (TCEP added just prior to use from a 1 M stock solution in water).

Substrate: DDIVPCSMSYTW, 25 μM final concentration (from a 2 mM stock solution in DMSO stored at -20°C to avoid oxidation).

Tracer: reduced mono iodinated substrate biotin DDIVPC $SMS[^{125}I Y]TW$ (~1 nM final concentration).

HCV NS3 protease type 1b, 25 nM final concentration (from a stock solution in 50 mM sodium phosphate, pH 7.5, 10% glycerol, 300 mM NaCl, 5 mM DTT, 0.01% NP-40).

NS4A Cofactor peptide: KKGSVVIVGRIILSGRK, 2.5 μM final concentration (from a 2 mM stock solution in DMSO stored at -20°C).

B. Protocol

- The assay was performed in a 96-well polypropylene plate. Each well contained:
 - \circ 20 μ L substrate/tracer in assay buffer;
 - \circ 10 μ L \pm inhibitor in 20% DMSO/assay buffer;

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 $\circ~10~\mu L$ NS3 protease 1b/NS4 cofactor peptide (molar ratio 1:100).

Blank (no inhibitor and no enzyme) and control (no inhibitor) were also prepared on the same assay plate.

The enzymatic reaction was initiated by the addition of the enzyme/NS4A peptide solution and the assay

mixture was incubated for 40 min at 23°C under gentle agitation. Ten (10) µL of 0.5N NaOH were added and

10 µL 1 M MES, pH 5.8 were added to quench the enzymatic reaction.

15 Twenty (20) μL of avidin-coated agarose beads (purchased from Pierce[®]) were added in a Millipore[®] MADP N65 filtration plate. The quenched assay mixture was transferred to the filtration plate, and incubated for 60 min at 23°C under gentle agitation.

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The plates were filtered using a Millipore $^{\otimes}$ MultiScreen Vacuum Manifold Filtration apparatus, and 40 μ L of the filtrate was transferred in an opaque 96-well plate containing 60 μ L of scintillation fluid per well.

The filtrates were counted on a Packard® TopCount instrument using a ¹²⁵I-liquid protocol for 1 minute.

30 The value of IC_{50} was calculated in the same manner as in Example 19.

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Example 21

SPECIFICITY ASSAYS

- The specificity of the compounds was determined

 against a variety of serine proteases: human
 leukocyte elastase, porcine pancreatic elastase and
 bovine pancreatic α-chymotrypsin and one cysteine
 protease: human liver cathepsin B. In all cases a

 96-well plate format protocol using a colorimetric p-
- nitroanilide (pNA) substrate specific for each enzyme was used. Each assay included a 1 h enzyme-inhibitor pre-incubation at 30°C followed by addition of substrate and hydrolysis to ≈30% conversion as measured on a UV Thermomax® microplate reader.
- Substrate concentrations were kept as low as possible compared to K_M to reduce substrate competition. Compound concentrations varied from 300 to 0.06 μM depending on their potency. The final conditions for each assay were as follows:
- 50mM Tris-HCl pH 8, 0.5 M Na₂SO₄, 50 mM NaCl, 0.1 mM EDTA, 3% DMSO, 0.01% Tween-20 with;
 [100 μM Succ-AAPF-pNA and 250 pM α-chymotrypsin],
 [133 μM Succ-AAA-pNA and 8 nM porcine elastase], [133 μM Succ-AAV-pNA and 8 nM leukocyte elastase]; or
 [100 mM NaHPO₄ pH 6, 0.1 mM EDTA, 3% DMSO, 1mM TCEP, 0.01% Tween-20, 30 μM Z-FR-pNA and 5 nM cathepsin B (the stock enzyme was activated in buffer containing
- A representative example is summarized below for porcine pancreatic elastase:

20 mM TCEP before use)].

105

In a polystyrene flat-bottom 96-well plate were added using a Biomek[®] liquid handler (Beckman):

- $\circ~40~\mu L$ of assay buffer (50 mM Tris-HCl pH 8, 50 mM NaCl, 0.1 mM EDTA);
- 5 20 μL of enzyme solution (50 mM Tris-/HCl pH 8, 50 mM NaCl, 0.1 mM EDTA, 0.02% Tween-20, 40 nM porcine pancreatic elastase); and
 - 20 μL of inhibitor solution (50 mM Tris-HCl, pH 8,
 50 mM NaCl, 0.1 mM EDTA, 0.02% Tween-20, 1.5 mM-
- 10 0.3 μ M inhibitor, 15% v/v DMSO).

After 60 min pre-incubation at 30°C, 20 µL of substrate solution (50 mM Tris-HCl, pH 8, 0.5 M Na₂SO₄, 50 mM NaCl, 0.1 mM EDTA, 665 µM Succ-AAA-pNA) were added to each well and the reaction was further incubated at 30°C for 60 min after which time the absorbance was read on the UV Thermomax® plate reader. Rows of wells were allocated for controls (no inhibitor) and for blanks (no inhibitor and no enzyme).

The sequential 2-fold dilutions of the inhibitor solution were performed on a separate plate by the liquid handler using 50 mM Tris-HCl pH 8, 50 mM NaCl,

0.1 mM EDTA, 0.02% Tween-20, 15% DMSO. All other specificity assays were performed in a similar fashion.

The percentage of inhibition was calculated using the formula:

 $[1-((UV_{inh}-UV_{blank})/(UV_{ctl}-UV_{blank}))] \times 100$

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A non-linear curve fit with the Hill model was applied to the inhibition-concentration data, and the 50% effective concentration (IC_{50}) was calculated by the use of SAS software (Statistical Software System; SAS Institute, Inc., Cary, N.C.).

Example 22

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Tables of compounds

The following tables list IC_{50} values of compounds representative of the invention.

The following abbreviations are used:

15 IC₅₀: The concentration required to obtain 50% inhibition in the NS3 protease/NS4A cofactor peptide radiometric assay according to example 11; the results marked with an * indicate an IC50 value obtained in the recombinant HCV NS3 protease 20 radiometric assay according to example 10; HLE: The concentration required to obtain 50% inhibition in the human leukocyte elastase assay; PPE: The concentration required to obtain 50% inhibition in the porcine pancreatic elastase assay; 25 Other: Figures unmarked indicate the concentration required to obtain 50% inhibition in the bovine pancreatic α -chymotrypsin assay; figures marked with ** indicate the concentration required to obtain 50% inhibition in the human liver cathepsin B assay; MS: 30 Mass spectrometric data (MH from FAB); AAA: amino acid analysis data expressed in % peptide recovery; Acca: 1-amino-cyclopropylcarboxylic acid; Acpe: 1-

amino-cyclopentylcarboxylic acid; Abu: 2-aminobutyric

acid; Chg: cyclohexylglycine (2-amino-2-cyclohexyl-

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acetic acid); Hyp: 4(R)-hydroxyproline; Hyp(4-Bn):
4(R)-benzyloxyproline; Pip: pipecolic acid (i.e.
homoprolyl); Tbg: tert-butylglycine; Ac: acetyl; Bn:
benzyl; O-Bn: benzyloxy; DAD: 3-carboxypropionyl; and

DAE: 4-carboxybutyryl; AlGly: allylglycine (2-amino-4-pentenoic acid); thioxolle: L-thionoisoleucine; Ph:
phenyl; 3I-Ph: 3-iodophenyl; 4I-Ph: 4-iodophenyl;
2Br-Ph: 2-bromophenyl; 3Br-Ph: 3-bromophenyl; 4Br-Ph:
4-bromophenyl; 1-NpCH₂O: naphthalen-1-ylmethoxy; 2NpCH₂O: naphthalen-2-ylmethoxy 3,5-Br₂Ph: 3,5dibromophenyl.

		AAA	(%)	113	85.4 ± 1.6	100.3 ± 1.8	113.85 ± 4.9	95.8 + 0.8	98 8 + 2 E	00.0 ± 2.0	404 4F 1.1	101.15 ± 1.65	99.2 ± 5	102.95 ± 3.65		109.7 ± 6.9	72.4 ± 2.4	103.65 ± 3.8	59.4 + 2.R5	95.4 + 1.5
		MS	(MH.)	703	717	646	703	717	717	687	701	10/	600	67/	703	703	717	743	691	719
		Other	(mm)																	
		PPE	(ITM)																	
	م م	H	(mm)																	
	29 W	.	(mm)	40	26	000	0.0	1.5	16*	85*	31	*08	24*	70	6	35	.gc	50*	58	16*
ы	E E	P	٥	S S	S O		2	cys	Cys	Cys	Cys	Cys	Cvs	J.Ve	200	5	cys	Cys	Cys	Cys
TABLE	27	×	Pro	Pro	Pro	Pro	2 0	011	Pro	Pro	Pro	Pro	Pro	Pro	Pro	S d	2 6	Pro	Abu	Leu
	2 O= Z	РЗ	Val	Val	Val	Val	le/	٨٥١	vai	Vai	Val	Val	Val	Val	Val	9	2 2	615 ×	Val	Val
	z′ m	P4	<u>e</u>	le e	lle	<u>e</u>	<u>a</u>		≘	<u>e</u>	<u>=</u>	Val	Chg	Tbg	Leu	l e	2 2	2 2	2 2	9
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		9d	Asp	Glu	ŀ	Asp	Asp	Acn	de0 4	dsh.	Asp	Asp	Asp	Asp	Asp	Asp	Asn	Acn	200	dev
		B	Ac	Ac	DAD	Ac	Ac	Ac	2 2	2 2	₽.	Ac	Ac	Ac	Ac	Ac	Ac	Ac	2 0	2
		Compound	101	102	103	104	105	106	107	00	90	601	110	111	112	113	114	115	116	2

SUBSTITUTE SHEET (RULE 26)

	AAA	(%)	9.66	96.8 ± 1	87.0 ± 3.0	Z.S.	101	91.0 ± 4.5	107.6	106.3 ± 8.2	94.02 ± 3.19	100.2	107	100.9 ± 3.6	99.8 ± 0.6	107			
	MS	(MH.)	753	27.0	617	//0	600	083	669	697	711	683	713	713	269	642			
	Other	(mm)																	
	PPE	E E						000	>300										
ao ¥o	HLE	(MIN)						000	2300										
2g W	S) SE	133*	8 6	76*	17	315	220	040	017	017	45	cno	7.4	270*	123	24	36	
ق مر <u>ح</u>	P1	ر ا	SkS	Cvs	Cvs	Cvs	Abu	Nya	A SIGN	Sign of the second	aday	Acca	I NA	INVA	Nva	Nva	Cys	Acca	
2	×	Phe	Val	lle	Ala	Hyp(4-Bn)	Pro	Pro	Pro	D D	0.0		d - 0	2 2	Pro	Pro	Glu	Olu	
8g	РЗ	Val	Val	Val	Val	Val	Val	Val	Val	Val	//3	Na.	le/	3 3	\all	Vai	n Ö	Glu	
	P4	<u>e</u>	<u>≘</u>	≘	le	lle	=	<u>e</u>	<u> </u>	<u> </u>	<u>a</u>	9	9	2 =		9	S D D	Chg	
·	P5	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asn	Asp	D-G	T Po	500	2 0	<u> </u>	D-Glu	
	9 —	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Acn	3		Asb	Asp	
Ĺ	ω	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	A	DAN		Q Y	Ac	
	Compound	117	118	119	120	121	122	123	124	125	126	127	128	129	130	121	2	132	-

	AAA	(%)	
	MS	(MH+)	
	Other	(mm)	
	PPE	(mW)	
æ_ >= o	HE	(mm)	\int
29 W	<u>ြ</u>	39	
ξ	P1	Acca	
24	M	Glu(OBn)	
å	РЗ	Val	
z	P4	Chg	
	PS	Эlc	
	P6	Ac Asp	
	В	Ac	
	Compound	133	

SUBSTITUTE SHEET (RULE 26)

																	_
		AAA	(%)	107	103	96.3 ± 1.7	95		98.7	101.9		112	104	7	*	1017+54	
		MS	(MH.)	802	789	819	819		813	819		855	855	861	3	849)
		Other	(MIH)			>300**										×300	
		PPE	(mm)			>300			_					ŀ		>300	
	₩ -	HLE	(MIT)			>300										×300	T
2		<u>ကို</u>	7.2	200	3.0	0.0	9.4*	67.	;	6.4		0.39	0.71	2.6		0.033	
	.`o =o	P	No	Nys	NA V	BAN :	Nva	N		Nva	14	Nva	Nva	Nva		Cys	
2	-œ* 	R	O-Bn	O-Bn		10-0	o-tolyl- methoxy	m-tolvi-	methoxv	P-tolyl-	The methoxy	OZHONI-I	2-NpCH ₂ O	4-tert-butyl-	phenyl)- methoxv	O-Bn	
z	- cc °	РЗ	Val	Val	le/	3		\S		Val	3	2	Val	Val		Val	
zz		P4	lle	lle	9		e	lle		elle]	2 -	<u>e</u>	<u>e</u>		Chg	
		PS	Asp	D-Val	D-Glu	V	d S	Asp		Asp	Agn	3	ASD	Asp		D-Glu	
		P	Asp	Asp	Asp	Acn	2	Asp		Asp	Asn	2 2	Asb	Asp		Asp	
		m	Ac	Ac	Ac	Ac	2	Ac		Ac	Ac	2	3	Ac		Ac	
		Сошр.	201	202	203	204		205		506	207	208	000	60 7		210	
	P6 P5 P2 P3 P2 P3 P2 P3	B N N N N N N N N N N N N N N N N N N N	B N N N N N N N N N N N N N N N N N N N	В Р6 Р5 Р4 Р3 Р7	B P6 P5 P4 P3 P1 IC ₅₀ HLE PPE Other MS AC ASP D-Val He Val O-Bn Niva Octa	B P6 P5 P4 P3 P1 IC ₅₀ HLE PPE Other MS Ac Asp D-Val IIe Val O-Bn Nva 0.93 Ac Asp D-Clu IIe Val O-Bn Nva 0.93	B P6 P5 P4 P3 P2 R ₂ N N N N N N N N N N N N N N N N N N N	B P6 P5 P4 P3 P2 AC ASP ASP B-in N/a O-Bin N/a O-Go N/a O-Go N/a O-Bin N/a O-Go N/a N	B P6 P5 P4 P3 P2 B P6 P5 P4 P3 R13 P1 IC50 HLE PPE Other MS AC ASp ASp IIe Val O-Bn Nva 7.2 HLE PPE Other MS AC ASp D-Val IIe Val O-Bn Nva 0.63 A 789 AC Asp D-Glu IIe Val O-loly- Nva 0.65 >300 >300** 819 AC Asp Asp IIe Val methoxy Nva 9.4* 819 AC Asp Asp IIe Val m-tolyl- Nva 6.7*	B PG PS P4 P3 P1 IC ₅₀ HLE PPE Other MS AC ASp D-Val Ile Val O-lolyl- O-lolyl- Nva Nva 0.6 >300 >300 >300** 819 AC Asp Asp Ile Val O-lolyl- Nva 0.6 >300 >300 >300** 819 AC Asp Asp Ile Val O-lolyl- Nva 0.6 >300 >300 >300*** 819 AC Asp Asp Ile Val M-lolyl- Nva 0.6 >300 >300 >300*** 819 AC Asp Asp Ile Val methoxy Nva 6.7** R19 R19	B P6 P5 P4 P3 P2 AC ASp ASp P4 P3 R13 P1 IC ₅₀ HLE PPE Other MS AC ASp ASp IIe Val O-Bn Nva 7.2 HLE PPE Other MM1 AC ASp ASp IIe Val O-Bn Nva 0.6 >300 >300 789 AC ASp ASp IIe Val O-Bn Nva 0.6 >300 >300 819 AC ASp ASp IIe Val O-Ivjv- Nva 6.7* R19 AC ASp ASp IIe Val P-Ivjv- Nva 6.7* R19 AC ASp ASp IIe Val P-Ivjv- Nva 6.7* R19 AC ASp ASp IIe Val P-Ivjv- Nva 6.7*	B P6 P5 P4 P3 P2 AC ASP ASP ASP BFI3 R13 R13 P1 IC50 HLE PPE Other MS AC ASP ASP IIe Val O-Bn Nva 0.6 >300 >300 805 AC ASP ASP IIe Val O-Bn Nva 0.6 >300 >300 819 AC ASP ASP IIe Val IIe IIe Val	B P6 P5 P4 P3 R13 P1 C ₅₀ HLE PPE Other MS	B P6 P5 P4 P3 P2 AC ASP ASP	B P6 P5 P4 P3 R13 P1 IC ₅₀ HLE PPE Other MS Ac Asp Asp Asp Na O-10yl- Na 0.93 Chib Immethoxy R19 R1	B P6 P5 P4 P3 P1 IC ₅₀ P1 P1 AC ASP ASP ASP He Val O-Bn Nva 7.2 P1 IC ₅₀ P1 P1 IMM1 IMM1 <t< td=""><td>B P6 P5 P4 P3 P2 AC ASp ASp</td></t<>	B P6 P5 P4 P3 P2 AC ASp ASp

	444	¥ %	93.4 ± 2	99.4 + 2	101 B	104.1		:	100.6 ± 0.8	94.6 ± 3	111.2	95.7			S	N.S.
	W	(MH²)	845	803	698	895		879	789	818	910	740	269	683	869	737
	Other	(Mrl)				>300	>300**									
	PPE	(MII)		>300		>300										
o Ho	HE	(MIM)		^300		>300										
	<u>င်</u>	(mm)	0.12	0.21	0.036	0.028	1,00	0.014	3	E 6	0.43	2.3	31	22	8	21
	P		NVa	Acca	Nva	Nva	0000	Acca	EAN I	Na	2 2	NA	NVa	Nva	Nva	Nva
2	R ₁₃	a		10-10-14	Z-NPCH2O	2-NpCH ₂ O		Ba	100/40	O.B.	1-North		O2001	-NPCH2O	-NPCH2O	O ₂ HOdN-1
~	РЗ	\ \ 	- F	3	7	Val	Val	is	[e/	2 2	2 2	16/	3	2 2		٧٩
ź	P4	Cha			2 d	<u> </u>	Cha	e e	a	9	е			2 2	2 2	2
	PS	D-G	D-Glu	- C		ם מומ	D-Glu	Asp	Asp	D-Glu	Asp					
	P6	Asp	Asp	Asp	Aen	2	Asp	Asp	Asp	Asp	1	:	:			
	B	Ac	Ac	Ac	Ac		Ac	Ac	Ac	Ac	Ac	DAD	DAD	DAE	0=	
	Comp.	211	212	213	214		215	216	217	218	219	220	221	222	223	_

	AAA (%)	(o/)	N.S.		:	;							
	MS		737		929	707	635	613.4	9,0	818	0/0.4	929.2	
	Other												
	PPE (mM)												
. π. >= o _	HLE (LM)						>600	>600					
2 <u>E. S </u>	1C ₅₀		56	AE.	32.0	0.70	8	35	2.2	200	4.	0.14	
	F		Nva	Nico	2000	Acca	Acca		o ex	Acca	Acca	Acca	
2	R ₁₃		1-NpCH ₂ O	1-NnCH.O	OF HOUSE		I-NPCH2O	O-Bn	Ph(CH ₃),	1-NoCH ₂ O	1-NpCH ₂ O	(31-	ר טקרטנווי
~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	РЗ		Val	\Z	//3	3 2	BA	Val	Val	Cha	Chg	Val	
	P4		<u>e</u>	9	Ę	200	<u> </u>	S S S	lle	Cha	Chg	lle	
	P5			:	:			:	Asp	:	i	Glu	
	P6		!	:	:			:	Asp	<u> </u>	i	Asp	
	8		٠=	Ac	DAE	Δ	2 2	¥	Ac	Ac	AcOCH ₂ -C(0)	Ac	
	Сошр.		224	225	226	227	300	077	230		•	233	

	Γ		T	T	T		T		T	T	T	1		_	$\overline{}$	
	444	\{ \(\)	(%)													
	SM	(MH2)			720	(M+M)	598	(M±Na)	75.							
	Other	(mm)														
	PPE	(MI)														
. Æ >= o	HE	(mM)							195							
Z Z Z	<u>င်</u>	(MH)	41	12	4.0		5.5		27	27	42	18		36	35	1
	P1		Acca	Acca	Nva		Acca		Acca	Acca	Acca	Acca		Acca	Acca	 1
2	R ₁₃		ua-o	-NPCH20	I-NPCH2O		OZHODNI-I	770-07/	Old-Pay	(28r-Pn)0	Olu-ras	___	\s__\s__\	(4Br-Ph)S	<u></u>	
~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	РЗ	240	3 6	5 2	ا ا ا	19/	ਰ ^	16/	200	C C	2	ਰ >		Val	Val	
	P4	PHO C		thiovolla	all-ovollis	oll o	2	Cha	2 5	200	2 5		Š	5 2		
	P5	!	:	2€	î	:			ŀ		:					
	P6	!	:	!		:		!	:	;	:					
	8	Ac	Вос	Ac		DAE		Ac	Ac	Ac	Ac		Ac	Ac	!	
	Сотр.	234	235	236		237		238	239	240	241		242	243		-

	AAA	(%)	
	MS	(MH)	
	Other	(hind)	
	PPE	(min)	
- 4	HLE		
2 <u>R.</u> N	IC _{So}	10	5.0
	P1	Acca	Acca
2	R	رمي م	- 5°
≥ °= Z	РЗ	Val	Val
	P4	Chg	Chg
	P5	i	ı
	P6	1	ł
	1	Ac	Ac
	Сошр.	244	245

	* * * *	(%)			119±1		
	S	(MH²)		803.6	0.000.0		
	Other	(Mn)					
	PPE	(MI)					
. °. >=°	HE	(MM)					
/	ج ا ق	(mw)		9	3.6		9.7
E E	P	Acca		Nva	Acca		Acca
2	R	OMe)— 0	Ph(CH ₂) ₂	CH,O	Ž	(4I-Ph)O
م محر	P3	Val		Val	Chg		Val
Z	P4	Chg	-	9	Chg		င် ရ
	P5	i	V	Asb	;		:
	P6	!	V	dec	:		:
	m	Ac	Ac		Ac	Q	2
	Сошр.	246	247	070	049	249	

	_								
	AAA	(%)					91±1		
	MS	(MH.)					651.4		-
	Other	(mm)			-				
	PPE	(MII)							
. e⁻ >=o	HLE	(MI)							
Z	C _{S0}	(MIM)	. સં	13	2	S	3 8	88	
E E	P1	V	Acca	Arra		N	800	Acca	
2	R ₁₃			0(0)2 ОН	I _z	1-NoCH.O	2	о но(о)о	
o=\z	Р3	16/	5	Val		Val	//	3	
z. m	P4	Cha		Cha		Cha	Cha	, ,	
	PS	:		:		:	:		
	9d	:		:		:	:		
	В	Ac		Ac	į	Ac	Ac		
	Comp.	250		251		252	253		

_		118		
AAA	(%)			
MS	(MH¹)			
Other	(mm)			
PPE	(MIM)			
HE	(Mu)			
<u>ာ</u>	ξ.1		4.5	-
4	Acca		Acca	Acca
R ₁₃		MeC(O)		S 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
P3	Val		Val	Val
P4	Chg		වි ට	Chg
P5	l		1	i
P6	!		ŀ	I
6	Ac		S.	Ac
Comp.	254	330	665	- 256
	5. B P6 P5 P4 P3 R ₁₃ P1 IC ₅₀ HLE PPE Other MS	B P6 P5 P4 P3 R13 P1 IC ₅₀ HLE PPE Ac Chg Val (μM) (μM) (μM) (μM)	B P6 P5 P4 P3 R13 P1 IC ₅₀ HLE PPE Other MS AAA Ac Chg Val OACa 5.1 IMM) (µM) (µM) (MH*) (%) MeClO) Meclo)	B P6 P5 P4 P3 R13 P1 IC ₅₀ HLE PPE Other MS AAA Ac Chg Val Odd Acca 5.1 (μM) (μM) (μM) (μM) (MH*) (%) Ac Chg Val Nec(o) Acca 4.5 Acca

	AAA						
	MS						631 (M+Na)
	Other						
	PPE (IIM)						
¥ 0	HLE	>300					
2 <u>R.</u> X	[K]	2.2	16	28	0.18	28	40
	P	Acca	Acca	Acca	Cys	Cys	Acca
2	R	المالية	Z S	J. J. J. W.	O-Bn	O-Bn	1-NpCH ₂ O
2 2 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	P3	Val	Val	Val	Val	Val	Val
z	P4	Chg	Chg	Chg	lle	Chg	Ee lee
	P5	:	:	:	D-Glu	:	:
	P6	!	:	ŀ	Asp	:	
	മ	Ac	Ac	Ac	Ac	Ac	Ac
	Сошр.	257	258	259	260	261	262

	_		20		*
	AAA	(%)			
	WS	(M+Na)	811	811	721.4
	Other	(M _H)			
	PPE	(MII)			
. a }=0	HE	(MM)			
2 <u>a.</u> z	So	(µМ)	6.4	10	9.7
	P1	Acca	Acca	Acca	Acca
2 EZ	R ₁₃	1-NpCH ₂ O	1-NpCH ₂ O	1-NpCH20	1-NpCH20
2	P3	Val	Val	Val	Val
z. m	P4	<u>=</u>	ell e	<u>=</u>	<u>•</u>
	P5	ŀ	i	i	1
	P6			i	:
	ω.	HOOC Me Me CO	8,8-5	8	S-8 8-8
	Сошр.	263	264	265	266

			•		
	AAA	(%)			
	MS	721.4	665.1	835.5 (M-H)	745 (M-H)
	Other	(him)			
	PPE	(mir)			
e >= o	HLE				
g <u>g.</u> <u>g.</u> <u>y</u>	IC _{So}	12	24	2.2	2.0
	P	Acca	Acca	Acca	Acca
2 EZ	R ₁₃	1-NpCH ₂ O	(3Br- Ph)CH ₂ O	1-NpCH ₂ O	1-NpCH ₂ O
~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	РЗ	Val	Val	Val	Val
	P4	<u>a</u>	Chg	Chg	Chg
	PS		i		:
	P6	.	;	!	i
		8	Ac	8 <u>,</u> 	HOO TO
	Comp.	267	268	569	270

	AAA (%)	
	MS (MH*)	
	Other (µM)	
	PPE (µM)	
	HLE (µM)	
= \	1C ₅₀ 3.8 3.8	27 17.5 7.6 6.2
	P1 Acca	Acca Acca Acca
2 = 0 2 = 0	R ₁₃ 1-NpCH ₂ O	(3,5-Br ₂ - Ph)CH ₂ O H
° ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° °	Val	Val Val
	Chg	Chg Chg
	5	Asp D-Val
	8 .	Asp Asp
	B	Ac Ac Ac
	Comp. 271	272 273 274 275

			.20			·
	AAA	(%)	8.66		102	104.4
	MS	(MH;)	713		713	753
	Other	(MIT)				
	PPE	(MI)				
FO HO	HLE	(MIM)				
2	. S	IMIT		•08	8	44.
E E-	F	N. S.		Nya	3	Nva
24 E	A		N Track	Mer	N 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	H _{III} O
o=\	P3	Val		Val		Val
	P4	<u>=</u>	!	<u>=</u>		<u>=</u>
	P5	Asp		Asp		Asp
	P6	Asp		Asp		Asp
	8	Ac		Ac		Ac
	Entry #	301		305		303
	P5 P4 P3 P2 P1 P1 P3 P2 P1	B P6 P5 P4 P3 P2 P1 B P6 P5 P4 P3 P2 P1 B P6 P5 P4 P3 P2 P1 B P6 P5 P4 P3 W R1 R1 B P6 P5 P4 P3 W P1 C ₅₀ HLE PPE Other MS	В Р6 Р5 Р4 Р3 Р2 Р1 В Р6 Р5 Р4 Р3 Р2 Р1 В Р6 Р5 Р4 Р3 W Р1 IC ₅₀ HLE РРE Other MS Ас Asp Asp IIe Val	Ac Asp A	B P6 P5 P4 P3 P2 P1 B P6 P5 P4 P3 P2 P1 Ac Asp Asp IIe Val (Met.) (Me	B P6 P5 P4 P3 P2 P1 Ac Asp Asp

	AAA (%)
	MS (MH*)
	Other (µM)
	PPE (µM)
4	HLE (µM)
	1.1 1.1
E E ZI	Acca
24	
0= Z	Val
"	Chg
P5	;
P6	I
æ	Ac
Entry	304

SUBSTITUTE SHEET (RULE 26)

	AAA (%)			
	MS (MH*)	747.4	761.4	783.3
	PPE (µM)			
	HLE (µM)			
	IC _{So}	7.9	28	9.6
H	<u>P</u>	Acca	Acca	Acca
2 <u>R. S</u> 2	R	1-NpCH ₂ O	1-NpCH ₂ O	1-NpCH ₂ O
- \ × o=(Œ,	cyclohexyl	cyclohexyl	cyclohexyl
<u>a </u>	P3	Va Va	Val	Val
	P5			
	9d			
		NOOC N	Меоос	-z
	Comp.	2	402	403
	B S S S S S S S S S S S S S S S S S S S	2. B P6 P5 P3 P2 B P6 P5 P3 P2 B P6 P5 P3 P4 B P6 P6 P7 P1 CμM) (μM) (μM) (μM) (μM) (μM) (μM) (μM) (B P6 P5 P3 P2 B P6 P5 P3 P2 B P6 P6 P6 P1 HOOC N N N OH N N N N N N N N N N N N N N N N N N N	B P6 P5 P3 P1 IC ₅₀ HLE PPE MS (μM) (μM) (μM) (μM) (μM) (μM) (μM) (μM)

	AAA	<u>%</u>			
	MS	(MH*) 797.3	721.4	735.3	
	PPE	(Mil)			
	HLE	(MIL)			
	<u>်</u>		0.8	25	
# 0	7	Acca	Acca	Acca	
	R ₁₃	1-NpCH ₂ O	1-NpCH ₂ O	1-NpCH ₂ O	
2	3	cyclohexyl	cyclohexyl	cyclohexyl	
	P3	Val	Val	Val	
	PS				7
	P6				
	æ	-x ®wooo	HOOC-CH ₂ CH ₂ - N(Me)C(O)-	MeOOC-CH ₂ - CH ₂ -N(Me)C(O)-	
i	Сошр.	404	405	406	

	AAA	(%)			
	MS	749.3	763.3	735.4	763.4
	PPE ("W)				
	HLE				
	IC _{So}	1.5	=	24	32
¥ o	19	Acca	Acca	Acca	Acca
2 <u>R. S. 2</u>	R ₁₃	1-NpCH ₂ O	1-NpCH ₂ O	1-NpCH ₂ O	1-NpCH ₂ O
ω ω σ ο σ ο σ ο σ ο σ ο σ ο σ ο σ ο σ ο	R	cyclohexyl	cyclohexyl	cyclohexyl	cyclohexyl
	РЗ	Val	Val	Val	Val
	PS				
	P6				
	©	HOOC-CH ₂ CH ₂ - N(CH(Me) ₂)- C(O)-	MeOOC-(CH ₂) ₂ - N(CH(Me) ₂)- C(O)-	HOOC-CH ₂ - N(CH(Me) ₂)- C(O)-	EtOOC-CH ₂ - N(CH(Me) ₂)- C(O)-
	Comp.	407	408	409	410

	AAA	8			
	MS	763.4	751.3	779.3	
	PPE	(Mrt)			
	HLE		-		
	IC _{So}	7.4	0.8	0.12	
₽	ā	Acca	Acca	Acca	
2	R ₁₃	1-NpCH ₂ O	cyclohexyl 1-NpCH ₂ O	1-NpCH ₂ O	
	ď	cyclohexyl	cyclohexyl	cyclohexyl	
	РЗ	Val	Val	Val	
	PS				
	P6				
	83	HOOC-(CH ₂) ₃ - N(CH(Me) ₂ -C(O)	[HOOC-CH ₂] ₂ - NC(0)-	[HOOC-(CH ₂) ₂] ₂ - NC(O)-	
	الم	114	412	413	

				<u> </u>
	AAA (%)			
	MS (MH*)	761.3	803.2	791.1
	PPE (µM)			
P2 N N N N N N N N N N N N N N N N N N N	HLE (µM)			
	IC _{so} (µM)	0.78	0.89	0.41
	P	Acca	Acca	Acca
	R ₁₃	1-NpCH ₂ O	1-NpCH ₂ O	cycloheyxl 1-NpCH ₂ O
	œ.	cyclohexyl	cyclohexyl	cycloheyxl
	РЗ	Val	Val	Val
	PS			
	P6			
	8			
	Сотр.	414	415	914

	AAA	(%)		
	MS	763.2	797.3	775.6 (M-H)
	PPE			
	HLE (#M)			
	(E. S.)		0.63	1.4
H _O	<u>a</u>	Acca	Acca	Acca
	R ₁₃	1-NpCH ₂ O	1-NpCH ₂ O	1-NpCH ₂ O
	œ	cyclohexyl	cyclohexyl	cyclohexyl
	P3	Val	Val	
	P5			
	P6			
			HO No	
	Comp.	4	418	419

			1	
	AAA (%)			
	MS (MH*)	925.6 (MK) [†]	841.5 (MK)*	778.4
	PPE (IIM)			
	HLE (µM)			
	1Cs0 (MH)	0.52	1.7	0.4
¥	<u>P</u>	Acca	Acca	Acca
M. N. O. M.	R ₁₃	cyclohexyl 1-NpCH ₂ O	1-NpCH ₂ O	1-NpCH ₂ O
	œ	cyclohexyl	cyclohexyl	cyclohexyl
	P3	Val	Val	Val
	PS			
	9d			
	æ	HO-CHCH ₂		HO Ne
	Сотр.	420	421	422

B R N N N N N N N N N N N N N N N N N N		AAA		ł		_
		MS	(MHT)	726.3		
		PPE	(mid)			
		HLE	(MI)			
		ာ လ	(mid)	7.9		
		£		Acca		
		R ₁₃		1-NpCH ₂ O Acca		
	6	ř		cyclonexyl		
	02	2	13	Val		
	PS					
	96	· 				
	6 0			IZ	=0	
	Comp.		423			

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What is claimed is:

1. A compound of formula (I):

P6 P5 P4 P3 P2 P1 $\begin{bmatrix}
H & O \\
N & H
\end{bmatrix}$ $\begin{bmatrix}
R_5 \\
N & O
\end{bmatrix}$ $\begin{bmatrix}
R_4 \\
N & H
\end{bmatrix}$ $\begin{bmatrix}
R_3 \\
N & H
\end{bmatrix}$ $\begin{bmatrix}
R_1 \\
N & H
\end{bmatrix}$ $\begin{bmatrix}$

wherein Q is CH_2 or N-Y, wherein Y is H or C_{1-6} alkyl;

a) when \mathbf{Q} is CH_2 , \mathbf{a} is 0, \mathbf{b} is 0, and \mathbf{B} is an amide derivative of formula $\mathbf{R_{11a}N(R_{11b})}$ -C(0)- wherein $\mathbf{R_{11a}}$ is H; C_{1-10} alkyl optionally substituted with carboxyl or di(loweralkyl) amino; C_{3-7} cycloalkyl; C_6 aryl; C_{7-10} alkylaryl; $(C_{3-7}$ cycloalkyl)- $(C_{1-6}$ alkyl); heterocycle- C_{1-6} alkyl;

and R_{11b} is C_{1-6} alkyl substituted with carboxyl, $(C_{1-6}$ alkoxy) carbonyl or phenylmethoxycarbonyl; or C_{7-16} aralkyl substituted on the aromatic portion with carboxyl, $(C_{1-6}$ alkoxy) carbonyl, phenylmethoxycarbonyl, or heterocycle- C_{1-6} alkyl;

or R_{11a} and R_{11b} are joined to form a 3 to 7-membered nitrogen-containing ring optionally substituted with carboxyl or (C_{1-6} alkoxy) carbonyl;

or

b) when Q is N-Y; a is 0 or 1, b is 0 or 1, and B is
25 an acyl derivative of formula R₁₁-C(0)-wherein R₁₁ is
(i) C₁₋₁₀ alkyl optionally substituted with carboxyl,
C₁₋₆ alkanoyloxy (e.g. AcOCH₂) or C₁₋₆ alkoxy (e.g.
Boc); (ii) C₃₋₇ cycloalkyl optionally substituted with carboxyl, (C₁₋₆ alkoxy)carbonyl or

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phenylmethoxycarbonyl; (iii) C_{3-7} cycloalkyl substituted with carboxyl and one to three C_{1-6} alkyl substituents (iv) C_{4-10} (alkylcycloalkyl) optionally substituted on the cycloalkyl portion with carboxy, $(C_{1-6}$ alkoxy)carbonyl or phenylmethyoxycarbonyl; (v)

HOOC-(
$$C_{1-6}$$
alkyl)-N NCOO-(aryl or C_{1-6} alkylaryl) ,

(v) C_6 or C_{10} aryl or $C_{7\text{--}16}$ aralkyl optionally substituted with $C_{1\text{--}6}$ alkyl;

 $\mathbf{R_6}$, when present, is C_{1-6} alkyl substituted with carboxyl; and

 R_5 , when present, is C_{1-6} alkyl optionally substituted with carboxyl;

or

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15 c) when Q is either CH_2 or N-Y;

 R_4 is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl);

Z is oxo or thioxo;

 $\mathbf{R_3}$ is $C_{1\text{--}10}$ alkyl optionally substituted with

carboxyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl); W is a group of formula II:

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wherein $\mathbf{R_2}$ is C_{1-10} alkyl or C_{3-10} cycloalkyl optionally substituted with carboxyl; C_6 or C_{10} aryl or C_{7-16} aralkyl; or

W is a group of formula II':

wherein X is CH or N; and

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 R_2 ' is divalent C_{3-4} alkylene which together with X and the carbon atom to which X and R_2 , are attached form a 5- or 6-membered ring, said ring optionally substituted with OH; SH; NH₂; carboxyl; R_{12} ; OR₁₂, $C(0)OR_{12}$, SR_{12} , NHR_{12} or $NR_{12}R_{12}$ ' wherein R_{12} and R_{12} 'are independently:

cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl, 15 said alkyl or alkenyl optionally substituted with NH2, OH, SH, halo, or carboxyl; said alkyl or alkenyl optionally containing at least one heteroatom selected independently from the group 20 consisting of: O, S, and N; or $\mathbf{R_{12}}$ and $\mathbf{R_{12}}'$ are independently $\mathbf{C_6}$ or $\mathbf{C_{10}}$ aryl or C_{7-16} aralkyl optionally substituted with C_{1-6} alkyl, CF_3 , NH_2 , OH, SH, halo, carboxyl, C_{1-6} alkyl substituted with carboxyl, phenyl 25 optionally substituted with C_{1-6} alkyl, C_{1-6} alkoxy, halo, acetylamido or nitro; said aryl or aralkyl optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N; 30 said cyclic alkyl, cyclic alkenyl, aryl or

aralkyl being optionally fused with a second 5-,

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6-, or 7-membered ring to form a cyclic system or heterocyclic system, said second ring being optionally substituted with NH₂, OH, SH, halo, carboxyl or carboxy(lower)alkyl; said second ring optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N;

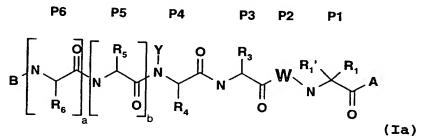
or X is CH or N; and R₂, is a divalent C₃₋₄ alkylene which together with X and the carbon atom to which X and R₂, are attached form a 5- or 6-membered ring which in turn is fused with a second 5-, 6- or 7-membered ring to form a cyclic system wherein the second ring is substituted with OR₁₂, wherein R₁₂, is C₇₋₁₆ aralkyl;

15 R_1 ' is hydrogen, and R_1 is C_{1-6} alkyl optionally substituted with thiol or halo; or R_1 is C_{2-6} alkenyl; or

 $R_1\prime$ and R_1 together form a 3- to 6-membered ring optionally substituted with C_{1-6} alkyl; and

A is hydroxy or a pharmaceutically acceptable salt or ester thereof.

2. A compound of formula (Ia):



25 wherein \mathbf{Y} is \mathbf{H} or \mathbf{C}_{1-6} alkyl;

a is 0 or 1;

b is 0 or 1;

 \boldsymbol{B} is an acyl derivative of formula $\boldsymbol{R_{11}}\text{-C(O)-wherein}$ $\boldsymbol{R_{11}}$ is (i) $C_{1\text{--}10}$ alkyl optionally substituted with

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carboxyl, C_{1-6} alkanoyloxy or C_{1-6} alkoxy; (ii) C_{3-7} cycloalkyl optionally substituted with carboxyl, (C_{1-6} alkoxy)carbonyl or phenylmethoxycarbonyl; (iii) C_{3-7} cycloalkyl substituted with carboxyl and one to three C_{1-6} alkyl substituents (iv) C_{4-10} (alkylcycloalkyl) optionally substituted on the cycloalkyl portion with carboxy, (C_{1-6} alkoxy)carbonyl or phenylmethyoxycarbonyl; (v)

HOOC-(
$$C_{1-6}$$
alkyl)-N NCOO-(aryl or C_{1-6} alkylaryl) ,

HO
$$(CH_2)_{1.5}$$
 or $(CH_2)_{1.5}$

10 (v) C_6 or C_{10} aryl or C_{7-16} aralkyl optionally substituted with C_{1-6} alkyl;

 \mathbf{R}_{6} , when present, is C_{1-6} alkyl substituted with carboxyl;

 \mathbf{R}_5 , when present, is C_{1-6} alkyl optionally substituted with carboxyl; and

 R_4 is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl);

 R_3 , W, R_1 , R_1 and A are as defined in claim 1.

20 3. A compound of formula Ia according to claim 2, wherein $\bf B$ is an acyl derivative of formula $\bf R_{11}C(0)$ - wherein $\bf R_{11}$ is:

 C_{1-6} alkyl optionally substituted with carboxyl, C_{1-6} alkanoyloxy or C_{1-6} alkoxy;

 C_{3-7} cycloalkyl optionally substituted with carboxyl, MeOC(O), EtOC(O) or BnOC(O); 3-carboxypropionyl (DAD) or 4-carboxybutyryl (DAE);

or

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HOOCCH₂N NCOOBn

4. A compound of formula Ia according to claim 3, wherein ${\bf B}$ is acetyl, 3-carboxypropionyl, 4-carboxylbutyryl, AcOCH₂C(O), Me₃COC(O),

A compound of formula Ia according to claim 4, wherein B is acetyl, 3-carboxypropionyl (DAD), 4 carboxybutyryl (DAE), AcOCH₂C(O),

$$C(O)$$
 or $C(O)$ $C(O)$

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- 6. A compound of formula Ia according to claim 5, wherein, **B** is acetyl.
- 7. A compound of formula Ia according to claim 2, wherein \mathbf{R}_6 , when present, is the side chain of Asp or Glu.
 - 8. A compound of formula Ia according to claim 7, wherein \mathbf{R}_6 , when present, is the side chain of Asp.
- 9. A compound of formula Ia according to claim 2, wherein \mathbf{R}_5 , when present, is the side chain of an amino acid selected from the group consisting of D-Asp, Asp, D-Glu, Glu, D-Val, Val, D-Tbg and Tbg.
- 10. A compound of formula Ia according to claim 9, wherein \mathbf{R}_5 , when present, is the side chain of D-Asp, D-Val or D-Glu.
- 20 11. A compound of formula Ia according to claim 10, wherein R₅, when present, is the side chain of D-Glu.
 - 12. A compound of formula (Ib):

wherein **B** is an amide of formula $\mathbf{R_{11a}N(R_{11b})C(0)}$ - wherein $\mathbf{R_{11a}}$ is C_{1-6} alkyl, C_{3-6} cycloalkyl, C_{3-7} (alkylcylcoalkyl) optionally substituted with carboxy, C_{1-3} carboxyalkyl, C_6 aryl, C_{7-10} arylalkyl, 2-tetrahydrofuranylmethyl, or 2-thiazolidylmethyl;

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and R_{11b} is C_{1-6} alkyl substituted with carboxyl.

- 13. A compound of formula (Ib) according to claim
- 12, wherein R_{11a} is cyclopropylmethyl, isopropyl,
- 5 carboxyethyl, benzylmethyl, benzyl, or 2-tetrahydrofuranylmethyl.
 - 14. A compound of formula (Ib) according to claim
 - 13, wherein R_{11b} is C_{1-4} alkyl substituted with
- 10 carboxyl.
 - 15. A compound of formula (Ib) according to claim
 - 14, wherein R_{11b} is ethyl carboxyl.
- 16. A compound of formula I according to claim 1, wherein R₄ is selected from the group consisting of: isopropyl, cyclopropyl, tert-butyl, 1-methylpropyl, or 2-methylpropyl.
- 20 17. A compound of formula I according to claim 16, wherein $\mathbf{R_4}$ is cyclopropyl or 1-methylpropyl.
 - 18. A compound of formula Ia according to claim 17, wherein \mathbf{R}_4 is cyclopropyl.
 - 19. A compound of formula I according to claim 1, wherein ${\bf Z}$ is oxo.
- 20. A compound of formula I according to claim 1,
 wherein R₃ is the side chain of Ile, allo-Ile, Chg,
 Cha, Val, Tbg or Glu.
 - 21. A compound of formula I according to claim 20, wherein $\mathbf{R_3}$ is the side chain of Val, Tbg or Chg.

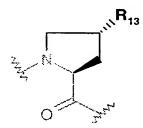
- 22. A compound of formula I according to claim 21, wherein $\mathbf{R_3}$ is the side chain of Val.
- 23. A compound of formula I according to claim 1, wherein \mathbf{W} is a group of formula II wherein \mathbf{R}_2 is C_{1-6} alkyl; C_{1-6} alkyl substituted with carboxyl, C_{1-6} alkoxycarbonyl, benzyloxycarbonyl or benzylaminocarbonyl; or benzyl.

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24. A compound of formula I according to claim 23, wherein \mathbf{W} is a group of formula II wherein $\mathbf{R_2}$ is the side chain of Abu, Leu, Phe, Cha, Val, Ala, Asp, Glu, Glu(OBn) or Glu (NHBn).

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- 25. A compound of formula I according to claim 24, wherein \mathbf{R}_2 is the side chain of Asp, aminobutyric acid (Abu) or Val.
- 20 26. A compound of claim I according to claim 1, wherein W is a group of formula III'



Formula III'

wherein R_{13} is OH; SH; NH₂; carboxyl; R_{12} ; OR₁₂, SR₁₂, NHR₁₂ or NR₁₂R₁₂' wherein R_{12} and R_{12} ' are

independently:

cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl, said alkyl or alkenyl optionally substituted with NH_2 , OH, SH, halo, or carboxyl; said alkyl

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or alkenyl optionally containing at least one heteroatom independently selected from the group consisting of: O, S, and N; or

R₁₂ and R₁₂, are independently C₆ or C₁₀ aryl or

C₇₋₁₆ aralkyl optionally substituted with C₁₋₆ alkyl, NH₂, OH, SH, halo, carboxyl or carboxy(lower)alkyl; said aryl or aralkyl optionally containing at least one heteroatom independently selected from the group consisting of: O, S, and N;

said cyclic alkyl, cyclic alkenyl, aryl or aralkyl being optionally fused with a second 5-, 6-, or 7-membered ring to form a cyclic system or heterocyclic system, said second ring being optionally substituted with NH₂, OH, SH, halo, carboxyl or carboxy(lower)alkyl; said second ring optionally containing at least one heteroatom independently

selected from the group consisting of: O, S, and N.

- 27. A compound of claim I according to claim 26, wherein R₁₃ is OR₁₂ or SR₁₂ wherein R₁₂ is a C₆ or C₁₀ aryl or C₇₋₁₆ aralkyl, said first aryl or aralkyl optionally substituted with C₁₋₆ alkyl, C₃₋₇ cycloalkyl, NH₂, OH, SH, halo, C₁₋₆ alkoxy, carboxyl, carboxy(lower)alkyl, or a second aryl or aralkyl; said first and second aryl or aralkyl optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N.
 - 28. A compound according to claim 27, wherein R₁₃ is Bn; PhCH₂CH₂; PhCH₂CH₂; O-Bn; o-tolylmethoxy; m-tolylmethoxy; p-tolylmethoxy; 1-naphtyloxy; 2-naphtyloxy; 1-naphthalenylmethoxy; 2-

naphthalenylmethoxy; (4-tert-butyl)methoxy; (3I-Ph)CH₂O; (4Br-Ph)O; (2Br-Ph)O; (3Br-Ph)O; (4I-Ph)O; (3Br-Ph)CH₂O; (3,5-Br₂-Ph)CH₂O;

29. A compound according to claim 28, wherein $\mathbf{R_{13}}$ is O-Bn; PhCH₂CH₂CH₂; 1-naphtyloxy; 2-naphtyloxy; 1-

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naphthalenylmethoxy; 2-naphthalenylmethoxy;

- 30. A compound of formula I according to claim 1, wherein $\mathbf{R_1}$, is hydrogen and $\mathbf{R_1}$ is C_{1-6} alkyl optionally substituted with thiol.
- 31. A compound of formula I according to claim 30, wherein $\mathbf{R_{1}}$, is the side chain of the amino acid selected from the group consisting of: cysteine (Cys), aminobutyric acid (Abu), norvaline (Nva), or allylglycine (AlGly).

- 32. A compound of formula I according to claim 31, wherein $\mathbf{R_{1}}'$ is H and $\mathbf{R_{1}}$ is propyl.
- 33. A compound of formula I according to claim 1, wherein $\mathbf{R_1}$, and $\mathbf{R_1}$ together form a 3- to 6-membered ring, said ring being optionally substituted with ethyl.
- 34. A compound of formula I according to claim 33, wherein $\mathbf{R_1}$, and $\mathbf{R_1}$ together form a cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl ring.

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35. A compound of formula I according to claim 34, wherein R_1 and R_1 together form a cyclopropyl ring optionally substituted with C1-6 alkyl.

- 5 36. A compound of formula I according to claim 1, wherein
 - a) \mathbf{Q} is CH_2 , \mathbf{a} is 0, \mathbf{b} is 0, and \mathbf{B} is an amide of formula $\mathbf{R_{11a}}N\left(\mathbf{R_{11b}}\right)-C\left(0\right)$ wherein $\mathbf{R_{11a}}$ is C_{1-6} alkyl optionally substituted with carboxyl, C_{3-6} cycloalkyl,
- 10 C_{3-7} (alkylcycloalkyl) optionally substituted with carboxy, (C_{1-3} alkoxy)carbonyl, phenyl, C_{7-10} arylalkyl, 2-tetrahydrofuranylmethyl, or 2-thienylmethyl;

and R_{11b} is (C₀₋₂ alkyl)phenyl optionally substituted

with carboxyl or (C₁₋₄ alkoxy)carbonyl; or C₁₋₆ alkyl substituted with carboxyl or (C₁₋₄ alkoxy)carbonyl; or R_{11a} and R_{11b} are joined to form a piperidine ring optionally substituted with carboxyl or (C₁₋₆ alkoxy)carbonyl;

20 or

- b) \mathbf{Q} is N-Y, wherein Y is H or C_{1-6} alkyl; \mathbf{a} is 0 or 1, \mathbf{b} is 0 or 1, and \mathbf{B} is an acyl derivative of formula $\mathbf{R_{11}}$ - \mathbf{C} (0) wherein $\mathbf{R_{11}}$ is (i) C_{1-6} alkyl, C_{1-6} alkyl substituted with carboxyl, MeC(0)0-, MeO-, EtO-
- , MeCH₂CH₂O- or Me₃C-O-; (ii) cyclopentyl or
 cyclohexyl optionally substituted with carboxyl; (iv)
 C₄₋₁₀ (alkylcycloalkyl) optionally substituted on the
 cycloalkyl portion with carboxyl;
 (v)

(vi) phenyl, benzyl or phenylethyl;

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 $R_6,$ when present, is $\rm CH_2COOH~or~CH_2CH_2COOH,$ $R_5,$ when present, is $\rm C_{1-6}$ alkyl or $\rm CH_2COOH~or~CH_2CH_2COOH;~or~}$

5 c) when \mathbf{Q} is either CH_2 or $\mathbf{N-Y}$,

 $\mathbf{R_4}$ is C_{1-6} alkyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl);

Z is oxo or thio;

 R_3 is C_{1-6} alkyl; C_{3-7} cycloalkyl or C_{4-10}

10 (alkylcycloalkyl);

W is a group of formula II wherein R_2 is C_{1-10} alkyl, C_{3-10} cycloalkyl, C_{7-11} aralkyl; CH_2COOH or CH_2CH_2COOH ; or W is a group of formula II' wherein X is N or CH and R_2 , is the divalent radical $-CH_2CH_2CH_2-$ or -

- CH₂CH₂CH₂CH₂- which together with \mathbf{X} and the carbon atom to which \mathbf{X} and \mathbf{R}_2 , are attached form a 5- or 6-membered ring, said ring optionally substituted with OR₁₂, C(0)OR₁₂, SR₁₂, NHR₁₂ or NR₁₂R₁₂, wherein R₁₂ and R₁₂, are independently:
- 20 cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl, said alkyl or alkenyl optionally substituted with NH2, OH, SH, halo, or carboxyl; said alkyl or alkenyl optionally containing at least one 25 heteroatom independently selected from the grop consisting of: O, S, and N; or R_{12} and $R_{12},\ \mbox{are}$ independently C_6 or C_{10} aryl or $C_{7\text{--}16}$ aralkyl optionally substituted with C_{1-6} alkyl, CF_3 , NH_2 , OH, SH, halo, carboxyl, C_{1-6} alkyl substituted 30 with carboxyl, or phenyl optionally substituted with C_{1-6} alkyl, C_{1-6} alkoxy or halo; said aryl or aralkyl optionally containing at least one heteroatom independently selected from the group

consisting of: O, S, and N; said cyclic alkyl,

cyclic alkenyl, aryl or aralkyl being optionally fused with a second 5-, 6-, or 7-membered ring to form a cyclic system or heterocyclic system, said second ring being optionally substituted with NH₂, OH, SH, halo, carboxyl or C₁₋₆ alkyl substituted with carboxyl; said second ring optionally containing at least one heteroatom independently selected from the group consisting of: 0, S, and N; or X is N; and R_2 , is - $CH_2CH_2CH_2$ or $-CH_2CH_2CH_2CH$ which together with ${\bf X}$ and the carbon atom to which X and R_2 , are attached form a 5- or 6-membered ring, which in turn is fused to a phenyl to form a cyclic system wherein the phenyl ring is substituted with OR12.. wherein R12.. is phenylmethyl or phenylethyl;

 R_1 , is hydrogen and R_1 is methyl, thiomethyl, 1-methylethyl, propyl, 1-methylpropyl, 2- (methylthio)ethyl or 2-propylene; or R_1 , and R_1 together with the carbon atom to which they are attached form a cyclopropyl which may optionally be substituted with ethyl; and A is hydroxy or a pharmaceutically acceptable salt thereof; C_{1-6} alkoxy, or (aryl C_{1-6} -alkoxy).

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37. A compound of formula Ia according to claim 2, **B** is an acyl derivative of formula R_{11} -C(0) - wherein R_{11} is C_{1-6} alkoxy, C_{1-10} alkyl optionally substituted with carboxyl; C_{3-7} cycloalkyl optionally substituted with carboxyl or benzylcarboxy; or

R₆ is absent;

R₅ is absent;

 R_4 is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl);

5 $\mathbf{R_3}$ is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl);

W is a group of formula II:

Formula II

wherein $\mathbf{R_2}$ is C_{1-6} alkyl; C_{3-6} cycloalkyl; C_{1-6} alkyl substituted with carboxyl; C_6 or C_{10} aryl; or C_{7-11} aralkyl;

or

W is a group of formula II':

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Formula II'

wherein \boldsymbol{X} is N; and $\boldsymbol{R_2}$, is as defined in claim 2, and

A is hydroxy or a pharmaceutically acceptable salt thereof; methoxy, ethoxy, phenoxy, or benzyloxy.

38. A compound of formula Ia according to claim 2, wherein **B** is acetyl, 3-carboxypropionyl, 4-

25 carboxylbutyryl, $AcOCH_2C(O)$, $Me_3COC(O)$,

Y is H or Me, a is 0 or 1, b is 0 or 1,

 R_6 , when present, is the side chain of Asp or Glu, R_5 , when present, is the side chain of Asp, D-Asp, Glu, D-Glu, Val, D-Val or Tbg,

;

 R_4 is the side chain of Val, Chg, Tbg, Ile or Leu, R_3 is hydrogen or the side chain of Ile, Chg, Val, Glu;

W is Abu, Leu, Phe, Val, Ala, Glu, or Glu(OBn); or
10 W is group of formula III':

wherein R_{13} is Bn, PhCH₂CH₂, PhCH₂CH₂CH₂, O-Bn, o-tolylmethoxy, m-tolylmethoxy, p-tolylmethoxy, 1-

naphthalenylmethoxy, 2-naphthalenylmethoxy, (4-tert-butyl) benzyloxy, (3I-Ph)CH₂O, (4Br-Ph)O, (2Br-Ph)O, (3Br-Ph)O, (4I-Ph)O, (3Br-Ph)CH₂O, $(3,5-Br_2-Ph)$ CH₂O,

 $R_{1}\text{, is }H\text{ and }R_{1}\text{ is the side chain of Cys, Abu, Nva or allylglycine; or$

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 R_1 , and R_1 together with the carbon atom to which they are attached form a cyclopropyl; and A is hydroxyl.

39. A compound of formula Ib according to claim 12, wherein and **B** is an amide of formula $R_{11a}N(R_{11b})-C(0)$ wherein R_{11a} is C_{1-6} alkyl optionally substituted with carboxyl, C_{3-6} cycloalkyl, C_{3-7} (alkylcycloalkyl) optionally substituted with carboxy, (C_{1-3} alkoxy)carbonyl, phenyl, C_{7-10} arylalkyl, 2-

tetrahydrofuranylmethyl, or 2-thienylmethyl; and R_{11b} is (C₀₋₂ alkyl)phenyl optionally substituted with carboxyl or (C₁₋₄ alkoxy)carbonyl; or C₁₋₆ alkyl substituted with carboxyl or (C₁₋₄ alkoxy)carbonyl; or R_{11a} and R_{11b} are joined to form a piperidine ring

optionally substituted with carboxyl or $(C_{1-6}$ alkoxy) carbonyl;

R4 is cyclohexyl,

Z is oxo;

 $\mathbf{R_3}$ is hydrogen or the side chain of Ile, Chg, Val, Glu;

W is Abu, Leu, Phe, Val, Ala, Glu, Glu(OBn); or

W is group of formula III':

wherein R₁₃ is Bn, PhCH₂CH₂, PhCH₂CH₂CH₂, O-Bn, o-tolylmethoxy, m-tolylmethoxy, p-tolylmethoxy, 1-naphthalenylmethoxy, 2-naphthalenylmethoxy, (4-tert-butyl)methoxy, (3I-Ph)CH₂O, (4Br-Ph)O, (2Br-Ph)O, (3Br-Ph)O, (4I-Ph)O, (3Br-Ph)CH₂O, (3,5-Br₂-Ph)CH₂O,

 $R_{1}\text{, is }H\text{ and }R_{1}\text{ is the side chain of Cys, Abu, Nva or allylglycine; or$

- R_1 , and R_1 together with the carbon atom to which they are attached form a cyclopropyl; and A is hydroxyl.
 - 40. A compound of formula I according to claim 1, wherein ${\bf B}$ is an acyl derivative of formula ${\bf R_{11}}\text{-C}(0)$ -

wherein R_{11} is C_{1-10} alkyl optionally substituted with carboxyl; C_{3-7} cycloalkyl optionally substituted with carboxyl; or a C_{4-10} (alkylcycloalkyl) optionally substituted on the cycloalkyl portion with carboxyl;

or R_{11} is C_6 or C_{10} aryl or $C_{7\text{--}16}$ aralkyl optionally substituted with a $C_{1\text{--}6}$ alkyl

a is 0 or 1;

 $R_{6}, \mbox{ when present, is } C_{1\text{-}6} \mbox{ alkyl optionally substituted}$ with carboxyl;

10 **b** is 0 or 1;

 R_5 , when present, is C_{1-6} alkyl optionally substituted with carboxyl;

Q is N-Y, and Y is H or C_{1-6} alkyl;

 $\mathbf{R_4}$ is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10}

15 (alkylcycloalkyl);

Z is oxo,

 $\mathbf{R_3}$ is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl);

W is a group of formula II:

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Formula II

wherein $\mathbf{R_2}$ is C_{1-6} alkyl; C_{1-6} alkyl optionally substituted with carboxyl; C_6 or C_{10} aryl; or C_{7-16} aralkyl;

W is a group of formula II':

Formula II'

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wherein X is CH or N; and

 R_2 ' is C_{3-4} alkyl that joins X to form a 5- or 6-membered ring, said ring optionally substituted with OH; SH; NH_2 ; carboxyl; R_{12} ; OR_{12} , SR_{12} , NHR_{12} or $NR_{12}R_{12}$ ' wherein R_{12} and R_{12} ' are independently:

cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl, said alkyl or alkenyl optionally substituted with NH_2 , OH, SH, halo, or carboxyl; said alkyl or alkenyl optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N; or

 R_{12} and R_{12} ' are independently C_6 or C_{10} aryl or C_{7-16} aralkyl optionally substituted with C_{1-6} alkyl, NH_2 , OH, SH, halo, carboxyl or C_{1-6} alkyl substituted with carboxyl; said aryl or aralkyl optionally containing at least one heteroatom selected independently from the group consisting

of: O, S, and N;
said cyclic alkyl, cyclic alkenyl, aryl or
aralkyl being optionally fused with a second 5-,
6-, or 7-membered ring to form a cyclic system
or heterocyclic system, said second ring being
optionally substituted with NH₂, OH, SH, halo,

carboxyl or carboxy(lower)alkyl; said second ring optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N;

30 and

 R_1 ', is hydrogen, and R_1 is C_{1-6} alkyl optionally substituted with thiol, or C_{2-6} alkenyl; or R_1 ' and R_1 together form a 3- to 6-membered ring optionally substituted with C_{1-6} alkyl; and

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A is hydroxyl or a pharmaceutically acceptable salt or ester thereof.

41. A pharmaceutical composition comprising an antibepatitis C virally effective amount of a compound of formula I of claim 1, or a therapeutically acceptable salt or ester thereof, in admixture with a pharmaceutically acceptable carrier medium or auxiliary agent.

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- 42. A method of treating a hepatitis C viral infection in a mammal by administering to the mammal an anti-hepatitis C virally effective amount of the compound of formula I of claim 1, or a
- 15 therapeutically acceptable salt or ester thereof.
 - 43. A method of inhibiting the replication of hepatitis C virus by exposing the virus to a hepatitis C viral NS3 protease inhibiting amount of
- 20 the compound of formula I of claim 1, or a therapeutically acceptable salt or ester thereof.
- 44. A method of treating a hepatitis C viral infection in a mammal by administering thereto an anti-hepatitis C virally effective amount of a combination of the compound of formula I of claim 1, or a therapeutically acceptable salt or ester

thereof, and an interferon.

30 45. The use of a compound of formula I of claim 1 for the treatment of a hepatitis C infection in a mammal comprising administering thereto an antihepatitis C virally effective amount of the compound of formula I.

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46. The use of a compound of formula I of claim 1 for the manufacture of a mdeicament for treatment of a hepatitis C infection in a mammal.

WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: C07K 14/18, 5/103, 5/107, 5/083, 5/078, A61K 38/16, 38/04

A3

(11) International Publication Number:

WO 99/07733

(43) International Publication Date:

18 February 1999 (18.02.99)

(21) International Application Number:

PCT/CA98/00765

(22) International Filing Date:

10 August 1998 (10.08.98)

(30) Priority Data:

60/055,186

11 August 1997 (11.08.97)

US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report: 20 May 1999 (20.05.99)

(54) Title: HEPATITIS C INHIBITOR PEPTIDES

(57) Abstract

Compound of formula (I) active against the Hepatitis C virus, wherein when Q is CH2, a is 0, b is 0 and B is an amide derivative; or when Q is N-Y wherein Y is H or C_{1-6} alkyl, then B is an acyl derivative; R_6 , when present, is C_{1-6} alkyl substituted with carboxyl; R_5 , when present, is C_{1-6} alkyl optionally substituted with carboxyl;

when Q is either CH₂ or N-Y, then Z is oxo or thioxo; R_4 is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl); R_3 is C_{1-10} alkyl optionally substituted with carboxyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl); W is a proline derivative; R_1 ' is hydrogen, and R_1 is C_{1-6} alkyl optionally substituted with thiol; or R_1 is C_{2-6} alkenyl; or R_1 ' and R_1 together form a 3- to 6-membered ring; and A is hydroxy or a pharmaceutically acceptable salt or ester thereof.

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A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C07K14/18 C07K IPC 6 C07K5/103 C07K5/107 C07K5/083 C07K5/078 A61K38/16 A61K38/04 According to International Patent Classification (IPC) or to both national classification and IPC **B. FIELDS SEARCHED** Minimum documentation searched (classification system followed by classification symbols) IPC 6 C07K A61K Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) C. DOCUMENTS CONSIDERED TO BE RELEVANT Category ' Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No. MORI E.A.: "The N-terminal region of NS3 X 1-9, 16,serine protease of HCV is important to 17, maintain its enzymatic integrity" 19-22, BIOCHEM.BIOPHYS.RES COMM., 36,37, vol. 231, no. 3, 24 February 1997, pages 40-46 738-742, XP002086571 see page 740, column 2 P,X LLINAS-BRUNET M ET AL: "Peptide-based 1 - 46inhibitors of the hepatitis C virus serine protease" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, vol. 8, no. 13, 7 July 1998, page 1713-1718 XP004137115 see the whole document -/--Χ Further documents are listed in the continuation of box C. X Patent family members are listed in annex. Special categories of cited documents: "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the "A" document defining the general state of the art which is not considered to be of particular relevance invention earlier document but published on or after the international "X" document of particular relevance; the claimed invention filing date cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another "Y" document of particular relevance; the claimed invention citation or other special reason (as specified) cannot be considered to involve an inventive step when the document is combined with one or more other such docudocument referring to an oral disclosure, use, exhibition or ments, such combination being obvious to a person skilled in the art. other means document published prior to the international filing date but later than the priority date claimed "&" document member of the same patent family Date of the actual completion of the international search Date of mailing of the international search report 17 March 1999 01/04/1999 Name and mailing address of the ISA **Authorized officer** European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Groenendijk, M Fax: (+31-70) 340-3016

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Category °	Action) DOCUMENTS CONSIDERED TO BE RELEVANT			
	Citation of document, with indication, where appropriate, of the relevant passages		Relevant to claim No.	
Ρ, Χ	WO 98 17679 A (DEININGER DAVID D ;MURCKO MARK A (US); VERTEX PHARMA (US); FARMER) 30 April 1998 cited in the application see the whole document		1-46	
, x	INGALLINELLA E.A.: "Potent peptide inhibitors of human HCV NS3 protease are obtained by optimizing the cleavage products" BIOCHEMISTRY, vol. 37, no. 25, 23 June 1998, pages 8906-8914, XP002086572 cited in the application see the whole document		1-46	
	LANDRO E.A.: "Mechanistic role of NS4A peptide cofactor with the truncated NS3 protease of HCV: elucidation of the NS4A stimulatory efect via kinetic mapping and inhibitor mapping" BIOCHEMISTRY, vol. 36, no. 31, 5 August 1997, pages 9340-9348, XP002086573 see the whole document		1-46	

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Box I	Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This Inte	ernational Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. X	Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely: Remark: Although claims 42-45 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. X	Claims Nos.: 1-37,40-46 partially because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
	see FURTHER INFORMATION sheet PCT/ISA/210
3.	Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II	Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This Int	ernational Searching Authority found multiple inventions in this international application, as follows:
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2.	As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
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4.	No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Rema	The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 1-37,40-46(all partially)

The claims are unduly broad and speculative, allowing substitutions in every position and lacking any significant constant structural entity. Therefore a meaningful and economically feasible search could not encompass the complete subject-matter of the claims. Consequently the search has been directed to the exemplified compounds and closely related analogs (and their use) and extended to compounds having the same activity and has been complete for the subject-matter of the claims 38 and 39 (Art.17(2)(a)(ii) and (b) PCT, PCT Guidelines CIII,2.1 and CIII,3.7).

Information on patent family members

PCT/CA 98/00765

Publication Patent family member(s) Patent document Publication cited in search report date date WO 9817679 30-04-1998 ΑU 5147798 A 15-05-1998

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